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CONTENTS OF Vol. XXVI, No. 4

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THE HISTOLOGY AND PHYSIOLOGY OF ROTENOIDS IN SOME PAPILIONACEAE. I

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(With Plates XXXV–XXXVIII and 12 Text-figures)

CONTENTS

INTRODUCTION

PRELIMINARY work by Worsley & Nutman (1937) showed that histological methods can be used in studying the distribution of rotenone and allied substances in *Derris elliptica*. This paper reports its extension to members of the Dalbergieae and the Galegeae, the tribes that include genera of insecticidal value. I have therefore abandoned the former main heading of this series of papers, which was originally intended to be confined to *Derris* and *Mundulea*.

The species and plant parts listed in Table I have been available for study.

Table I. *Species and plant parts available for study*

Tribe	Genus	Species	Parts available for study
Galegeae	*Mundulea*	*sericea** Chev.	All parts
	Tephrosia	*Vogelii* Hook.f.	All parts
		candida DC.	All parts
		Ehrenbergiana Schweinf.	All parts
		toxicaria Pers.	All parts
		macropoda Harv.	Seeds and seedlings only
		densiflora Hook.f.	All parts except seeds
		rigida Bak.	Roots, stems, leaves
		purpurea Pers.	All parts
		congestiflora Harms.	Roots, stems, leaves
	Millettia	*dura* Dunn.	All parts
		Bussei Harms.	Seeds only
		Stuhlmanii Taub.	Seeds only
		usaramensis Taub.	Seeds only
Dalbergieae	*Derris*	*uliginosa*† Benth.	All parts
		elliptica Benth.	All parts except flowers and seedlings
		Malaccensis Prain	All parts except flowers and seedlings
		polystachya Benth.	All parts
		dalbergioides Bak.	All parts
	Lonchocarpus	*Bussei* Harms.	All parts except roots and flowers
		Capassa Rolfe.	Seeds only

* Although I am not satisfied that two species of *Mundulea* do not exist, I am using the specific name *sericea*, rather than *suberosa*, in conformity with present taxonomic practice.

† I have been informed that this African species is now known as *Derris trifoliata* Lour.

Procedure and terminology

Details of the application of Durham's test to histological work are given by Worsley & Nutman (1937). In brief, the method consists of treatment with nitric acid followed by ammonia solution of thick sections cut from fresh material with a moistened microtome knife: cells containing rotenone and some allied substances show a fugitive blue or green colour. Also, in certain tissues where such cells are morphologically distinct and can be recognized in sections cut from wax-embedded material, 6 μ sections have been used in this study.

I have, hitherto, used the term "rotenone" to include all those naturally occurring substances which answer to Durham's test. This nomenclature is unsatisfactory, because confusion may arise between the actual substance rotenone and the group included in my extension of the meaning of the word, while with plants containing little or no rotenone, such as *Tephrosia Vogelii*, it is inappropriate. I shall, therefore, use the word Rotenoids,[1] which I define as those naturally occur-

[1] Purists might insist that rotenonoids is etymologically desirable: but I prefer rotenoids on the grounds of euphony and convenience.

ring substances which give a blue or green colour when treated with strong (about 75 %) nitric acid followed by strong ammonia solution.

For convenience, I shall refer to "rotenoid-cells" rather than to "rotenoid-containing cells", and to morphologically "distinct" rather than "specialized" cells. In the following descriptions all rotenoid cells are to be assumed to be not morphologically distinct unless otherwise stated.

Part I. The distribution of rotenoids in the plant parts

I have studied all available parts of plants at all stages of maturity. Consequently, the presentation of the data in any systematic form is difficult and involves some repetition. A statement of general distribution appears in Table II, while a more detailed account follows.

Table II. *General distribution of rotenoids in the plant parts*

Name	Country of origin	Habit	Insecticidal value	Summary of tissues found to contain rotenoids
Mundulea sericea	Widespread in Africa	Tree	Moderate	Roots, stems, peduncles, ovaries, stamens, seeds
Tephrosia Vogelii	Africa—cultivated	Shrub	Moderate	Throughout the plant
T. Ehrenbergiana	Widespread in Africa	Herb	Unknown	Roots, stems, sepals, petals, ovules, stamens, seeds
T. candida	Tropical Asia	Shrub	Poor	Roots, stems, ovules, seeds
T. toxicaria	Tropical America	Shrub	Moderate	Roots, stems, sepals, petals, ovules, anthers, ovaries, seeds
T. macropoda	South Africa	Recumbent shrub	Moderate	Roots, stems, petioles, seeds
T. densiflora	West Africa	Shrub	Unknown	Roots, stems, petals
T. rigida	East Africa	Shrub	Unknown	None
T. purpurea	Tropical Africa	Herb	Slight	Roots, seeds
T. congestiflora	East Africa	Shrub	Unknown	None
Millettia dura	East Africa	Tree	Unknown	Roots, stems, seeds
M. Bussei	East Africa	Tree	Unknown	None
M. Stuhlmanii	East Africa	Tree	Unknown	None
M. usaramensis	East Africa	Tree	Unknown	Seeds
Derris uliginosa	General Tropics	Creeper	Unknown	Roots, stems, petioles, seeds
D. elliptica	Malay and Burma	Creeper	High	Roots, stems, seeds
D. malaccensis	Malay and Burma	Shrub	High	Roots
D. dalbergioides	Malay and Burma	Tree	Nil	Traces in seeds
D. polystachya	Himalayas	Shrub	Nil	None
Lonchocarpus Bussei	East Africa	Tree	Nil	None

A. *Stems*

Mundulea sericea.

Rotenoids can first be detected when the young plant is 5–6 weeks old, in the expanded ends of the medullary rays and occasionally in the cortex. Suberization is subsequent to rotenoid deposition, and I am satisfied that no causal relationship connects the two processes. Additional rotenoid cells later occur in the rays and the cortex, and afterwards in the pith. Finally, rotenoids can also be found in the secondary rays and in the phloem.

Mundulea stems become woody at a very early age, and rotenoids are then confined to the rays, the phloem and the cortex (Pl. XXXV, fig. 1), and occasionally in the medullary rays of the xylem: none is found in the almost completely lignified pith.

Because of the unavoidable thickness of the sections (necessary with Durham's test), it is sometimes difficult to identify the precise tissue that contains rotenoids: this is particularly so when sieve tubes are under examination. I have examined a very large number of sections of the phloem of *Mundulea*: rotenoids are often found in cells occurring among sieve tubes and similar in shape to them, and in at least two instances I have been able definitely to confirm their presence in a sieve tube with identifiable sieve-plates. I consider therefore that rotenoids occur in sieve tubes, and also possibly in companion cells.

A well-marked increase in rotenoids occurs at all nodes, beginning to be apparent when the leaf is developing and increasing until it is fully grown. In a young stem, 4 months old, the numbers of rotenoid cells found in three series of four 80 μ sections are shown in Table III.

Table III

	2–4 mm. below node				At the node				2–4 mm. above node			
	1	2	3	4	1	2	3	4	1	2	3	4
Pith	1	3	3	2	13	9	12	8	6	6	2	2
Phloem	2	2	2	2	3	3	1	3	1	3	1	3
Medullary ray and cortex	4	3	2	3	22	15	6	9	5	4	3	2
Total	7	8	7	7	38	27	19	20	12	13	6	7
Average	7				26				9·5			

Another similar stem gave averages of thirty-nine rotenoid cells at the node, twelve below it and fifteen above it. The increase is mostly in the pith, to a lesser extent in the medullary rays, and scarcely at all in the phloem. Later, when the xylem cylinder is well formed and most of the pith lignified, few rotenoid cells can be found in it: but the xylem is less lignified at the nodes, and increases in rotenoids occur here in the pith, the cortex and the rays. In mature trees the nodes on the main stems still contain more rotenoid cells than do the internodes. This increase is confined to the medullary rays and the cortex, since the pith is completely lignified and contains no rotenoids. As the pith in these nodes must originally have contained rotenoids the evidence for lability of these compounds is good, especially since they persist in dead tissues, and can be recognized therein.

Most of the rotenoid cells in *Mundulea* stems are above the average cell size for the tissues in which they occur: but since they are not larger than the maximum it is not generally possible to distinguish them in cleared sections. Rotenoids and starch have never been found in the same cell.

Tephrosia Vogelii.

The stems of this species become woody at a later stage than those of *Mundulea*, and the lignification of the pith is likewise postponed. The deposition of rotenoids is similar in most respects to that in *Mundulea*. They occur mainly in the medullary rays, but some also in the cortex and in the pith: those in the pith, unlike *Mundulea*,

tend to occur at its periphery. Because of the delayed lignification of the pith, rotenoid cells persist therein longer than in that of *Mundulea*.

Rotenoid cells are again concentrated at the nodes, and the main increase occurs in the pith.

None of the cells is morphologically distinct, and rotenoids and starch do not occur in the same cells.

Tephrosia Ehrenbergiana.

I have been unable to obtain any young plants of this species. Young stems of mature plants contain, in an 80 μ section, an average of one to two rotenoid cells, confined to the pith. Below the first branch, four or five cells occur in each similar section.

In this plant there is no doubt that rotenoids are first laid down in the pith; only in older stems can they be found in the cortex, where they tend to occur opposite the main medullary rays.

There is an appreciable increase in the number of rotenoid cells at the nodes, particularly in the cortex of main stems just below the point of branching: one cell was detected in a xylem medullary ray. In smaller stems, the internodes of which contain no rotenoids in the cortex, the increase at the nodes is confined to the pith, with only an occasional rotenoid cell in the cortex: a count showed an average of twelve cells per section in the internode and twenty-two at the node.

Tephrosia candida.

Only a very few rotenoid cells, confined to the medullary rays of the phloem and not morphologically distinct, could be found in the stems of this species.

Tephrosia toxicaria.

The larger stems contain a fair number of rotenoid cells in the pith and medullary rays of the phloem, and a few in the cortex. Small stems do not contain rotenoids.

Tephrosia densiflora.

A few rotenoid cells occur in the cortex and phloem of this species, frequently in the phloem rays. There does not appear to be any increase in the number at the nodes.

Derris elliptica.

Young stems of *D. elliptica* contain no rotenoids, but in an established plant, with one or more vines, many resin cells occur in the pith, and in the basal portion many of these also contain rotenoids. In a vine 360 cm. long, from a 2-year-old plant, rotenoid cells occurred in the lower 45 cm., the number gradually diminishing from the base up to this point. In vines 400 cm. long, from a 3-year-old plant, rotenoids were found up to 60 cm. from the base. They occur almost entirely in the pith, rarely in the phloem and cortex, and then only near the base of the stem.

In longitudinal sections the resin and rotenoid cells are often seen in chains: Pl. XXXV, fig. 2 illustrates this; nearly all the cells in the lower half of the photograph contain rotenoids, although the continuity is broken by occasional resin cells

containing no rotenoids. None of the cells is morphologically distinct; they can only be differentiated with certainty by Durham's test. The contents of the rotenoid cells are frequently more globular.

In the previous paper (Worsley & Nutman, 1937) it was stated that rotenoids could not be found in the cuttings that were used. While this was true for that material (which was all obtained from the upper, rotenoid free stems), it is not always so, for cuttings from the rotenoid-containing basal portions of *Derris* stems contain rotenoids which persist. This original rotenoid content remains if the cuttings die; or if the apical part dies, as sometimes happens, there is no evidence that the rotenoids are translocated from it.

As the plant grows, rotenoids are developed in the pith, whether any were already present there or not.

A woody cutting contained forty to fifty rotenoid cells per section, 80 μ thick, in the pith only: when it had developed a vine 110 cm. long it was found to contain seventy to eighty such cells per section, also only in the pith. A shoot also contained a fair number, and the base of the vine a few.

Subsequently, rotenoid cells can be found in the phloem, but only in the portions below soil level. Here, also, numerous resin cells occur in the medullary rays of the xylem, and occasionally one of these contains rotenoids.

An increase in the number of rotenoid cells at the nodes is rare.

Derris uliginosa.

The stems of this species grow to a great length, vines 7 m. long being not uncommon. Rotenoid cells occur in the pith in moderate quantity along almost the whole length of the stems. In stems about 7 m. long, sections 80 μ thick show about forty rotenoid cells per section in the first 50–100 cm. from the base. The number slowly diminishes up the stem; at 5 m. it is about eight to ten per section, at 6 m. four or five and at 6·5 m. only one or two; at 20 cm. from the top only an occasional cell can be detected. At the base of the stem a fair number of rotenoid cells also occur in the cortex, but the colour with Durham's test is very fugitive, which probably indicates a low rotenoid content in each cell. The cortical rotenoid cells also diminish in number up the stem, and cease at 2–2·5 m., except at the nodes. An occasional rotenoid cell can also be found in the phloem.

At the nodes of main branching there is an increase of rotenoids in the cortex: thus in one stem where they had ceased in the internodes at about 250 cm. up, they were present in moderate number around a main branching 350 cm. up.

The rotenoid cells are isolated and not in chains as with *D. elliptica*.

Millettia dura.

Woody stems of this species contain a few rotenoid cells in the cortex: none has been detected in either pith or phloem, nor in young stems.

B. *Roots*

Mundulea sericea.

Rotenoid deposition in the root is very similar to that in the stem; but rotenoids do not appear until after the completion of the xylem cylinder, and are rarely found in the pith. The medullary rays and cortex contain more rotenoid cells than do the

corresponding parts of the stem (Pl. XXXV, fig. 3). In young roots many rotenoid cells are filled with small globules (Pl. XXXV, fig. 4).

The number of rotenoid cells increases at the points of branching, this increase being entirely confined to the rays and the cortex.

Tephrosia Vogelii.

Rotenoid deposition in these roots is very similar to that in *Mundulea*. Rotenoid cells appear at a slightly earlier stage and more are found in the pith, these latter tending to be peripheral, as in the stems. An occasional rotenoid cell can be found in the xylem parenchyma, but most occur in the medullary rays, as may be seen in Pl. XXXV, fig. 5.

Tephrosia Ehrenbergiana.

Only a few rotenoid cells occur in these roots, mostly in the cortex, rarely in the phloem. In fine roots a few are found in the cortex, but not in any particular position, such as opposite the medullary rays. I have not found any in roots prior to suberization.

Tephrosia macropoda.

The roots of seedlings contain rotenoid cells in the cortex opposite the medullary rays, and a few in the rays. No larger roots were available for examination.

Tephrosia toxicaria.

In young roots rotenoids first occur in the cortex opposite the protoxylem and gradually spread to the xylem parenchyma and especially the medullary rays. Older roots contain appreciably more rotenoid cells than do other *Tephrosia* species.

Tephrosia candida.

A few rotenoid cells occur in the cortex and phloem: no particular arrangement of these cells, such as opposite the protoxylem, is apparent.

Tephrosia purpurea.

Rotenoids are first detected in the cortex opposite the protoxylem in young roots, thereafter spreading to the xylem parenchyma and around the cortex. Medium-sized roots contain appreciable quantities, but production at this stage slows down and large roots therefore contain a comparatively small proportion of rotenoid cells.

Tephrosia densiflora.

In young roots rotenoids are first detected in the cortex, approximately opposite the protoxylem. In older roots a moderate number of rotenoid cells occur in the cortex and phloem.

Derris elliptica.

For completeness, I repeat briefly the earlier findings on *Derris* roots.

Until suberization has begun no rotenoids can be detected. They first occur in the secondary cortex opposite the protoxylem and primary medullary rays when the plant is about 6 weeks old. Thereafter they gradually spread throughout the cortex

and xylem parenchyma, especially in the medullary rays. In a mature plant all the xylem parenchyma cells appear to contain either rotenoids or starch, but never both in the same cell.

Derris malaccensis.

Rotenoid formation in these roots resembles that in *D. elliptica*, and does not take place prior to suberization. Although not so definitely as in *D. elliptica*, rotenoids occur first in the cortex opposite the medullary rays, and gradually spread throughout the cortex and xylem parenchyma; but the number of the rotenoid cells is always appreciably less than in that species.

Derris uliginosa.

Initial deposition of rotenoids in the cortex opposite the protoxylem is very obvious in this species: all roots which I have examined have been tetrarch, and the second stage of rotenoid formation is in the cortex opposite the rays which form between the primary rays, i.e. at eight points, as illustrated in Text-fig. 1. Thereafter, rotenoid cells occur all around the cortex in moderate number; but only a few scattered ones appear in the medullary rays, even in large roots. This species therefore contains considerably fewer rotenoids in its roots than *D. elliptica* or *D. malaccensis*.

In young plants, rotenoids besides occurring opposite the protoxylem are found in the cortex around the points where rootlets branch off.

Millettia dura.

A few rotenoid cells occur in woody roots in the cortex only: non-woody roots do not contain any.

C. *Nodules*

Although leguminous these plants rarely produce nodules: possibly only when there is a nitrogen deficiency in the soil.

I have occasionally found a rotenoid cell in the cortex of a nodule from normally grown *Derris elliptica* and *Mundulea*. *Derris elliptica* plants grown in sand culture with a nitrogen deficiency produce large numbers of nodules, most of which contain a few rotenoid cells in the cortex and in the phloem.

D. *Petioles and leaves*

Tephrosia Vogelii.

Rotenoid cells occur in the petioles, usually in the pith, and occasionally in the phloem and cortex. No increase occurs where the petiolules branch off, and rotenoid cells are only rarely found in the latter. The rotenoid cells are above the average, but not appreciably above the maximum cell size of the tissue in which they occur.

The mid-ribs of the leaflets contain rotenoids in moderate amount: at the basal end of a mature leaflet about every other 80 μ section contains at least one rotenoid cell, and some contain several (Pl. XXXV, fig. 6). The number of such cells diminishes along the mid-rib, and near the apex only about one section in six contains rotenoids. The vascular bundle of the mid-rib is surrounded by a sheath of sclerenchyma, often but sometimes not quite entire. Between the xylem and the adaxial sclerenchyma there are sometimes non-lignified cells about the size of the collenchymatous cells,

and frequently one of these contains rotenoids: usually however rotenoids occur only in the collenchyma.

Numerous, large, morphologically distinct rotenoid cells occur in the leaflet blades. They measure about $60 \times 50 \times 40\,\mu$ as compared with $20 \times 15 \times 15\,\mu$ for normal cells, i.e. 27 times as large, and lie with their longest side parallel with the mid-rib. They generally occur towards the adaxial side of the spongy mesophyll: Pl. XXXV, fig. 7, shows a transverse section through a mature leaflet.

The proportion of rotenoid cells increases with the maturity of the leaflet. Some are present from the earliest stages, and large, morphologically distinct rotenoid cells, about 20 times the volume of the surrounding ones (i.e. $30 \times 33 \times 37\,\mu$ as compared with $11 \times 11 \times 15\,\mu$), can be found in the plumule of an imbibed, but ungerminated,

Text-fig. 1.

Text-fig. 2.

Text-fig. 1. Rotenoid deposition in *D. uliginosa* roots. ▷, protoxylem; ●, groups of rotenoid cells in the cortex.

Text-fig. 2. Distribution of rotenoid cells (black) in plumule of *T. Vogelii*.

seed (Text-fig. 2). As the leaves unfold the proportion of rotenoid cells increases, and in a seedling with two leaves the younger contains about half the number in the older, which itself contains about its full complement. The petioles and petiolules of these young seedlings contain no rotenoids.

Derris uliginosa.

I have found a few rotenoid cells in the pith and phloem of the petioles, but none in the leaves.

Other species.

No rotenoids have been detected in either petioles or leaves of any other species.

E. *Peduncles*

Rotenoid cells are fairly numerous in both *Mundulea sericea* and *Tephrosia Vogelii* at the junctions of the peduncles with the stem and with the flower. In the former species a few such cells can usually be found along the length of the peduncle, always

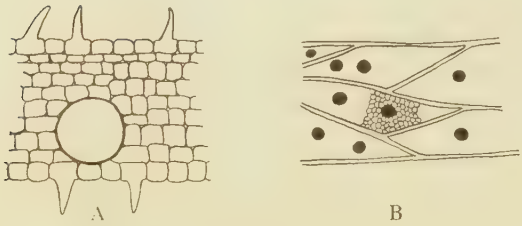
in the pith: the pith also containing most of the cells that occur at the junctions. In *T. Vogelii* considerably more rotenoid cells occur: these are mostly in the phloem, but some are also in the pith. Although sometimes appearing slightly swollen these rotenoid cells are not morphologically distinct.

I have not detected rotenoids in the peduncles of any other species.

F. *Sepals and petals*

Tephrosia Vogelii.

From a very early stage the sepals and petals contain rotenoid cells, confined to the parenchyma and most numerous towards the base of the organ. These are morphologically distinct, and are illustrated in Pl. XXXV, fig. 8 and Text-fig. 3.



Text-fig. 3.

Text-fig. 4.

Text-fig. 3. Rotenoid cells in sepals and petals of *T. Vogelii*. *A*, sepal, transverse: showing one large rotenoid cell. *B*, petal, longitudinal: rotenoid cells black.

Text-fig. 4. Distribution of rotenoid cells (black) in *T. Ehrenbergiana* petals.

In the sepals they are very constant in size, about 19 times that of the surrounding cells ($70 \times 80 \times 50\,\mu$ as compared with $25 \times 30 \times 20\,\mu$). In the petals they are 3–4 times as numerous as in the sepals and of the same size: but since the surrounding cells are smaller than in the sepals ($22 \times 27 \times 17\,\mu$), they are about 27 times the size of their neighbours.

Even in extremely young sepals and petals from small buds the morphologically distinct cells almost invariably contain rotenoids: it is therefore probable that they are not differentiated much in advance of rotenoid deposition. It will subsequently be shown that this is the opposite to what occurs in stamens and ovules.

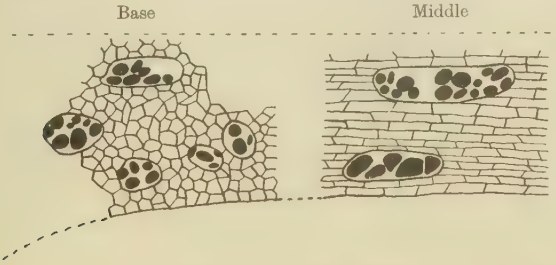
Morphologically distinct cells, identical with the rotenoid cells in appearance, occur in the sepals and petals, especially the latter. They contain an indicator pigment, colourless in untreated sections, turning magenta-red on addition of nitric acid, and becoming colourless again on further addition of ammonia: they never contain rotenoids. Similar cells occur in the ovary walls, in young green stems and occasionally in the bases of filaments.

Tephrosia Ehrenbergiana.

The petals and sepals of this species also contain morphologically distinct rotenoid cells, but unlike those in *T. Vogelii* they are long and narrow. In the petals their average size is $95 \times 30 \times 27\,\mu$ compared with normal cells varying between $30 \times 20 \times 25\,\mu$ and $45 \times 15 \times 15\,\mu$. They are more frequent near to the mid-rib, especially towards its basal end (Text-fig. 4). In one count they averaged thirteen per petal. In the sepals they are approximately the same size but are more scattered; they averaged twelve for a sepal twice the area of a petal.

Tephrosia densiflora.

A very few morphologically distinct rotenoid cells occur in the petals of this species: in twelve petals six such cells were found. They are about 21 times the volume of normal cells ($105 \times 52 \times 45\,\mu$ compared with $27 \times 22 \times 20\,\mu$). The sepals do not contain rotenoids.



Text-fig. 5. Rotenoid cells containing rotenoid globules (black) in the filament of *Mundulea*: longitudinal.

Tephrosia toxicaria.

About one morphologically distinct rotenoid cell per sepal can be detected, $4\frac{1}{2}$ times the size of normal cells ($110 \times 25 \times 22\,\mu$ compared with $50 \times 18 \times 15\,\mu$). One or two per petal can also be found, $3\frac{1}{2}$ times normal size ($150 \times 22 \times 22\,\mu$ compared with $60 \times 18 \times 18\,\mu$); the contents are globular.

G. *Stamens*

Mundulea sericea.

The cortical tissues of the filaments contain rotenoid cells which are frequent at the base, sparse along the length and moderately frequent at the apex.

Since the rotenoid cells are morphologically distinct and many times the size of their neighbours, they can be studied in sections prepared from embedded material. It is sometimes possible so to embed the material that the contents of these large cells remain intact. Pl. XXXVI, figs. 1, 2 show such cells in transverse sections of the base and the centre of a filament, and Text-fig. 5 longitudinal sections of similar cells. The globular nature of the contents is apparent: it can also be seen in sections of fresh material, and here all the individual globules react to Durham's test, except in sections from very young buds, when some of the globules are found not to contain rotenoids.

The rotenoid cells along the filament are longer and narrower than those in the base. The former are about 5 times the size of their neighbours ($95 \times 45 \times 40\,\mu$ compared with $55 \times 30 \times 20\,\mu$). Those in the base are about 40 times the size of normal cells ($85 \times 90 \times 60\,\mu$ compared with $25 \times 25 \times 17\,\mu$). Those in the apex are similar in size and shape to those in the base.

Rotenoid cells also occur in the connective tissue of the anther, and are about 80 times the volume of the surrounding cells ($85 \times 65 \times 65\,\mu$ as compared with $12 \times 15 \times 25\,\mu$). Their contents are often globular, but not so noticeably as in the cells of the filament (Pl. XXXVI, fig. 3).

In very small flower buds a few morphologically distinct cells can be detected in the connective tissue of the anthers and in the base of the filaments: these cells are considerably larger than the surrounding ones and contain globules of resins or oils, but no rotenoids. In slightly larger buds some of these cells contain rotenoids, but only a few of the globules in each cell sometimes answer the test. As the buds increase in size more rotenoid cells can be detected, including a few along the filament stem, and all their globules contain rotenoids.

It is thus established that the rotenoid cells in the stamens are predestined to contain rotenoids. In my experience some sections have shown that not all the morphologically distinct cells contain rotenoids, but there is no reason to suppose that all may not contain them at a later date.

Rotenoids are first detected in the anthers about the time when the pollen-grains become differentiated: I have not been able to detect them in any anthers before this process, but I have found anthers, in which the pollen-grains are differentiated, without rotenoids. The two phenomena occur at the same time, but no evidence for or against any direct relationship between them has been discovered.

Tephrosia Vogelii.

Morphologically distinct rotenoid cells occur in both filaments and anthers of this species. They are more frequent but relatively smaller than in *Mundulea*. At the base of the filament they measure about $60 \times 40 \times 50\,\mu$ as compared with normal cells of about $30 \times 15 \times 40\,\mu$, i.e. 7 times the size: along the filament length they are about 3 times the size ($50 \times 35 \times 45\,\mu$ compared with $15 \times 20 \times 85\,\mu$).

In the anthers they are about 40 times as large ($80 \times 50 \times 60\,\mu$ as compared with $20 \times 15 \times 20\,\mu$ for normal cells). Here however they vary considerably, and the above figures are only very approximate, but on the average they are smaller than in *Mundulea*. Pl. XXXVI, fig. 4 shows a transverse section of an anther.

As with *Mundulea*, the rotenoid cells are differentiated before rotenoids are deposited in them; but the pollen-grains are frequently formed before rotenoids can be detected.

Tephrosia Ehrenbergiana.

The anthers contain a larger proportion of rotenoid cells in their connectives than any other species that I have examined: these cells often appear as a chain along both sides of the connective. They are morphologically distinct and measure about $45 \times 45 \times 45\,\mu$, compared with normal cells of about $15 \times 12 \times 15\,\mu$, i.e. they are 34 times as large.

In the filaments, rotenoid cells occur in moderate numbers all along their length: in one batch examined the average number was twelve per filament. Their size is about $67 \times 30 \times 37\,\mu$, compared with about $90 \times 15 \times 22\,\mu$, or $2\frac{1}{2}$ times: they are wider but somewhat shorter than normal cells.

Tephrosia toxicaria.

The anthers contain a large number of morphologically distinct rotenoid cells about 10 times the size of the surrounding cells ($65 \times 27 \times 27\,\mu$ compared with $22 \times 15 \times 15\,\mu$): they occur in the connective tissue only, and are longer compared with their width than similar cells in other species. I have been unable to detect any rotenoids in the filaments.

Tephrosia candida, Derris uliginosa and *Millettia dura.*

Neither anthers nor filaments contain rotenoids, but morphologically distinct resin cells occur, especially in the connectives.

H. *Carpels and seeds*

Mundulea sericea.

Unfertilized ovules of this species normally contain three rotenoid cells which are always in about the same position. Three can be seen in longitudinal sections (see Pl. XXXVI, fig. 5) in which only the three dark cells in the integument contain rotenoids. Only two can be seen in transverse sections (Pl. XXXVII, fig. 1). These cells are morphologically distinct, as may be seen in Pl. XXXVII, figs. 1–3. They are at first filled with small globules, as in fig. 2, which later coalesce to form large ones, as in fig. 3.

In ovules from unopened buds the rotenoid cells are about 150 times as large as their neighbours ($75 \times 60 \times 35\,\mu$ compared with $15 \times 10 \times 7\,\mu$). Their longest side is parallel to the axis of the ovule, and they are more numerous and larger near the embryo-sac than elsewhere.

As with the anthers, the rotenoid cells are differentiated before they contain rotenoids, which are first demonstrable at the time of differentiation of the embryo-sac. It is not likely that there is any causal relationship between these two phenomena, since although I have never found rotenoids before differentiation of the embryo-sac, the converse is not always true.

Fertilization as such does not appear to affect the rotenoid cells, but concurrently with the subsequent growth of the ovule they increase in number, at first between the original cells and eventually all around the integument (Pl. XXXVII, fig. 2). The rotenoid cells are at first confined to the tissues that will eventually become the testa.

They remain of approximately the same size throughout the development of the testa, and are confined to the parenchyma. When the integument is fully developed they measure about $65 \times 55 \times 40\,\mu$, the largest seen being $80 \times 60 \times 60\,\mu$. In the dried mature seed they measure only $60 \times 40 \times 25\,\mu$, but return to their former size when swollen with water. The size of the neighbouring cells increases during the seed growth, and in a mature seed the rotenoid cells are only about 16 times the size of their neighbours.

Rotenoids do not occur in the cotyledons until they are definitely differentiated. Pl. XXXVII, fig. 4 is a section through an unripe but fairly mature seed: the

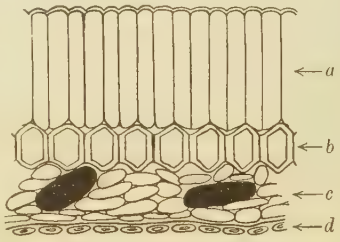
cotyledons although nearly fully developed contain no rotenoids. Pl. XXXVII, fig. 5 shows a mature seed with many rotenoid cells in the cotyledons. As the seed matures rotenoids are formed in the cotyledons, mainly in the peripheral palisade cells, many of which contain fixed oils. Rotenoids generally occur in an oil cell, although all oil cells do not contain rotenoids. In an unripe seed they are not distinguishable from the surrounding cells, but in a ripe seed they are generally somewhat larger than their neighbours, which often appear somewhat compressed: occasionally they are definitely larger than the average.

At the time rotenoids appear in the cotyledons they can also first be found in the radicle, but not in the plumule. They form a closely packed ring of cells in the peripheral cortex, and sometimes also around the central stele; rarely in the pith: Pl. XXXVII, fig. 5 illustrates a typical distribution. In transverse section, these cells appear as slightly swollen normal ones, but longitudinal sections show that they are morphologically distinct, being 2–3 times as long as their neighbours. The plumule does not develop rotenoid cells, but many occur in the cotyledons near the attachment.

The rotenone content of the ripe seed is 0·5–1 %: owing to the presence of about 14 % of fixed oils the determination is difficult. Fixed oils and proteins are the main food reserves: sugars and starch could not be detected.

Tephrosia Vogelii.

(*a*) *Ovules and seeds.* Rotenoid deposition follows closely that in *Mundulea*: morphologically distinct cells predestined to contain rotenoids can be detected in very small ovules, and rotenoids appear in them about the time of differentiation of the embryo-sac, although I have sometimes detected them earlier. Pl. XXXVII, fig. 6 shows large rotenoid cells in young ovules, and Pl. XXXVII, fig. 7 one such cell. The peculiar appearance of the contents is caused by shrinkage of the globules of rotenoid-containing resins. The relative sizes of the cells is well illustrated. Rotenoid cells average $80 \times 45 \times 75\,\mu$ compared with normal ones of about $30 \times 15 \times 18\,\mu$, i.e. they are 33 times as large.



Text-fig. 6. *T. Vogelii*, seed testa. *a*, palisade cells; *b*, column cells; *c*, parenchyma; *d*, aleurone cells. Two large rotenoid cells shown black.

As in *Mundulea*, rotenoids do not occur in the cotyledons until they are fully differentiated, when they also appear in the radicle, and in the plumule, especially towards its tip. Rotenoid cells are more scattered in these cotyledons than in *Mundulea*: they are not generally distinct morphologically, although many are slightly swollen, and a few are definitely larger than normal cells. Longitudinal sections frequently show distal accumulations of rotenoid cells.

Rotenoid cells are more generally distributed in the cortex and pith of the radicle than they are in *Mundulea*: in transverse sections they appear slightly swollen, but in longitudinal sections most are about twice the length of normal cells. In the plumule the rotenoid cells are morphologically distinct, as has been described on p. 657.

In the testa the rotenoid cells occur entirely in the parenchyma (Text-fig. 6),

which can be peeled off in strips: on treating these strips with Durham's reagents the rotenoid cells are readily seen (Pl. XXXVII, fig. 8).

The funicle does not contain rotenoid cells, but there is an increase in number in the integument where it joins.

Fixed oils and proteins form the main food reserves: sugars and starch are not present.

(*b*) *Ovaries.* From its earliest stages the ovary contains a few morphologically distinct rotenoid cells, generally situated towards the inner side of the spongy tissue of its walls (Pl. XXXVIII, fig. 1). They measure about $45 \times 45 \times 50\,\mu$, a few up to $150\,\mu$ long, compared with about $17 \times 17 \times 22\,\mu$ for normal cells, i.e. they are 16 times as large. As in the petals of this species, a number of morphologically distinct cells occur in the outer tissues of the ovary, containing an indicator pigment which turns red with acid and then becomes colourless on addition of ammonia.

(*c*) *Styles and stigma.* A few morphologically distinct rotenoid cells, four times the size of their neighbours, occur along the style, in the cortical tissue. Pl. XXXVIII, fig. 2 shows eight such cells (round and black), near the outer edges of a transverse section. The stigma does not contain any.

Tephrosia Ehrenbergiana.

Young ovules of this species do not contain rotenoids, which can only be detected in the fully differentiated cotyledons, and in the radicle. In the ripe seed a fair number of rotenoid cells occur in the cotyledons, particularly at both ends: the palisade cells rarely contain rotenoids. Although similar in outline to their neighbours they are about three times larger (about $60 \times 50 \times 45\,\mu$ compared with about $45 \times 35 \times 30\,\mu$). In the radicle rotenoid cells occur in the outer layers of the cortex, and also around the central stele: viewed transversely they appear as somewhat swollen normal cells, but longitudinally many of them are twice the length of normal ones, as if produced by fusion of two cells.

As would be expected from the absence of rotenoids in the young ovule, the testa contains none: nor does the plumule.

Fixed oils and proteins form the chief food reserves.

Tephrosia candida.

A moderate number of rotenoid cells occur in young ovules, in the integument. As in *T. Vogelii*, rotenoids occur prior to differentiation of the embryo-sac, and have even been detected in the youngest ovules obtainable. The rotenoid cells are 33 times the size of their neighbours ($60 \times 37 \times 40\,\mu$ compared with $15 \times 12 \times 15\,\mu$).

As the cotyledons become differentiated rotenoids appear, in normally sized cells, particularly in the sub-epidermal layer. In the radicles, rotenoid cells are similar to those in *T. Ehrenbergiana*, appearing transversely as somewhat swollen normal cells, but longitudinally about twice the length of such cells (Text-fig. 7).

A few rotenoid cells occur in the parenchyma of the testa: they are only slightly larger than the surrounding cells, and are not filled with rotenoids but contain isolated globules of them (Text-fig. 8).

The plumule does not contain rotenoids. The main food reserves are fixed oils and proteins.

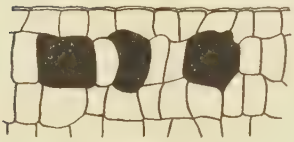
Tephrosia toxicaria.

(*a*) *Ovules and seeds.* A few morphologically distinct rotenoid cells can be detected in very small ovules from unopened buds: in ovules from open flowers a fair number occur, measuring about 43 times the size of normal cells ($37 \times 30 \times 22\,\mu$ compared with $10 \times 8 \times 7\,\mu$).

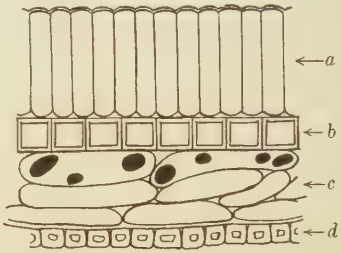
In imbibed mature seeds the testa contains rotenoid cells, in the parenchyma only, 24 times the size of the surrounding cells ($37 \times 22 \times 22\,\mu$ compared with $15 \times 7 \times 7\,\mu$).

Many large resin cells occur in the cotyledons and radicle: a fair number of slightly swollen normal cells containing rotenoids are also present. In the cotyledons the rotenoids are concentrated at each end, and in the radicle in the peripheral cortex especially at the abaxial side away from the cotyledons (Text-fig. 9).

Fixed oils and proteins are the chief food reserves.



Text-fig. 7.



Text-fig. 8.

Text-fig. 7. Longitudinal section through part of *T. candida* radicle: to show relative sizes of rotenoid cells (black).

Text-fig. 8. Seed testa of *T. candida*. *a*, palisade cells; *b*, colomn cells; *c*, parenchyma; *d*, aleurone cells. Rotenoid globules shown black.

(*b*) *Ovaries.* A few morphologically distinct rotenoid cells are found in the spongy tissue of the ovary, usually at the junctions of the two walls. They measure about $3\frac{1}{2}$ times the size of the surrounding cells ($30 \times 22 \times 22\,\mu$ compared with $18 \times 15 \times 15\,\mu$).

(*c*) *Styles.* A few rotenoid cells occur near the base only of the style: they are 3 times the size of normal cells ($30 \times 22 \times 22\,\mu$ compared with $37 \times 11 \times 11\,\mu$).

Tephrosia macropoda.

The following description is based on the only two seeds available.

The testa contains a fair number of morphologically distinct rotenoid cells in the parenchyma, about 29 times as large as their neighbours ($110 \times 60 \times 35\,\mu$ compared with $35 \times 15 \times 15\,\mu$).

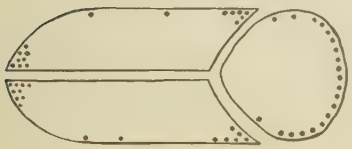
There are also a fair number of scattered normal sized rotenoid cells in the cotyledons, and the radicle contains rotenoids around its outer cortical layers, in cells frequently twice the length of normal ones, as if formed by fusion of two. The plumule is free from rotenoids.

Tephrosia purpurea.

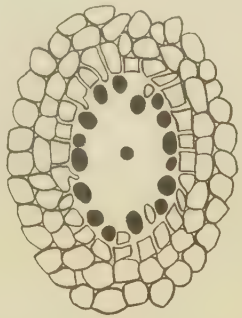
Only small and moderate-sized ovules were available: in the former no rotenoids occur. The cotyledons contain some rotenoid cells, which appear to be normal ones somewhat swollen. None occurs in the testa.

Tephrosia densiflora.

Only small ovules from buds and flowers, and nearly ripe seeds were available. The former contain no rotenoids: the latter have a fair number of rotenoid cells in the testa, $10\frac{1}{2}$ times the size of normal cells ($65 \times 52 \times 45\,\mu$ compared with $45 \times 22 \times 15\,\mu$). Rotenoids are slow in appearing in the cotyledons of this species: the fully differentiated cotyledons contain many resin cells, but not until the seed is nearly ripe do any of these develop rotenoids: at this stage the radicle has none: the cells are $1\frac{1}{2}$ times normal size ($37 \times 30 \times 30\,\mu$ compared with $30 \times 30 \times 22\,\mu$).



Text-fig. 9.



Text-fig. 10.

Text-fig. 9. Longitudinal section through ripe seed of *T. toxicaria*: to show distribution of rotenoid cells (black).

Text-fig. 10. Lysigenous cavity in *D. elliptica* seed: rotenoid globules black.

Derris elliptica.

This species has never flowered at Amani, but through the kindness of the Department of Agriculture of the Straits Settlements and Federated Malay States I obtained some seeds, but unfortunately none was viable. *Derris* seeds are very much larger than those of *Mundulea* or *Tephrosia Vogelii*, being $16 \times 9 \times 3$ mm. as compared with $5 \times 3 \times 1\frac{1}{2}$ and $6 \times 3\frac{1}{2} \times 2$ respectively. Their chief food reserve is starch, which occurs in transparent spherical grains, and not fixed oils. The cut seed, like the root, exudes a milky juice containing rotenoids and starch. Rotenoids occur in the cotyledons in large lysigenous cavities, formed by cell disintegration (Pl. XXXVIII, fig. 3 and Text-fig. 10). These are readily visible to the naked eye, averaging $300 \times 185 \times 135\,\mu$, the largest seen being $435 \times 230 \times 165\,\mu$, compared with normal cells of about $70 \times 45 \times 35\,\mu$, i.e. 68 times as large. Many of them are completely filled with rotenoid containing resins, but some only contain a globular coating around their walls. Occasionally an empty cavity was seen, but this may have been due to loss of its contents during sectioning. The radicles contain no rotenoids or cavities, but rotenoid cavities occur near the abaxial surfaces of the immature leaves of the plumule, averaging 76 times the size of the surrounding cells ($120 \times 70 \times 90\,\mu$ compared with $22 \times 15 \times 30\,\mu$). The available material was too shrivelled to photograph, but the appearance was very similar to Pl. XXXVIII, fig. 4.

Derris uliginosa.

I have only been able to obtain small ovules and half-ripe to fully ripe seeds: there is therefore a stage in the ovule growth which has not been examined.

In the small ovules no rotenoids can be detected, but a few morphologically distinct resin cells occur. In half-ripe seeds the cotyledons, which are already differentiated, contain large lysigenous cavities of rotenoids, very similar to those in *D. elliptica*: owing to the lack of material I am unable to say whether these rotenoids are formed before or after differentiation of the cotyledons, but on the analogy of other species it is probably afterwards. In addition to the many large cavities there are also a number of smaller ones. The large ones are 43 times the size of normal cells ($215 \times 145 \times 105\,\mu$ compared with $45 \times 45 \times 37\,\mu$).

The plumule contains similar cavities, 43 times the size of normal cells ($240 \times 100 \times 90\,\mu$ compared with $45 \times 37 \times 30\,\mu$): these occur near the abaxial surface of the immature leaves, and near the tip (Pl. XXXVIII, fig. 4).

The radicle does not contain rotenoids, but large rotenoid cavities generally occur in the cotyledons just below the junction; one measured $410 \times 150\,\mu$ in longitudinal section.

No rotenoids occur in the testa of the seed. Starch, and not fixed oils, is again the food reserve.

Derris dalbergioides.

Seeds of this species contain large cavities filled with resins in their cotyledons. A very faint and transient blue colour with Durham's test is sometimes obtained in a few of the cavities, indicating the presence of traces of rotenoids. No other parts of the seed answer to the test. The food reserve is starch.

Millettia dura.

Although this tree flowers prolifically it appears to set very few seeds: I have only been able to obtain small ovules from buds and flowers and a few ripe seeds, no intermediate stages being available.

Small ovules do not contain rotenoids. The seeds are large, about $12 \times 10 \times 2$ mm., and on removing the testa, which is free from rotenoids, long rotenoid cavities near the surface are plainly visible to the naked eye (Pl. XXXVIII, fig. 5). Sections show many large lysigenous cavities similar to those in *Derris* species, and apparently all of them contain rotenoids. The cavities average 27 times the size of surrounding cells ($200 \times 105 \times 105\,\mu$ compared with $60 \times 45 \times 30\,\mu$): some however are very much larger, and one of those visible in Pl. XXXVIII, fig. 5 measured $1575 \times 340 \times 250\,\mu$, or 1650 times the surrounding cell size.

The radicle also contains similar cavities of rotenoids mostly in the outer cortex: they are about 10 times normal cell size ($180 \times 160 \times 150\,\mu$ compared with $52 \times 37 \times 22\,\mu$: the largest seen measured $240 \times 180 \times 150\,\mu$).

The plumule contains rotenoid cavities immediately below the abaxial surface of the immature leaves: see Pl. XXXVIII, fig. 6, in which the cavities can be seen to have caused a localized expansion of the epidermis. The cavities are about 38 times the size of normal cells ($80 \times 50 \times 47\,\mu$ compared with $10 \times 10 \times 7\,\mu$).

Starch, and not oils, forms the main food reserve.

Millettia Bussei and *M. Stuhlmanii*.

The cotyledons of both these species, especially the former, contain large lysigenous cavities, but no rotenoids. Starch is the food reserve in both.

Millettia usaramensis.

Only a few old and shrivelled seeds of this species were available. Rotenoids were present in large cavities in the cotyledons and radicles: in the former they measured about 87 times the size of normal cells ($200 \times 105 \times 90\,\mu$ compared with $45 \times 22 \times 22\,\mu$). The radicle was too shrivelled for measurements to be made. Traces of rotenoids were apparent in the very shrivelled plumules.

Part II. Germination phenomena

The most interesting and important fact elicited during this investigation is the behaviour of rotenoids during germination. Unfortunately, no viable seed of *Derris elliptica* has yet been obtained.

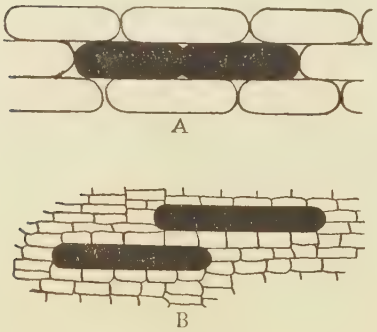
Mundulea sericea.

On germination the testa splits and is discarded, its rotenoids remaining in it undiminished. As the radicle elongates, additional rotenoid cells are formed throughout its length to the growing point. Those near the tip are generally 2–3 times the length of normal cells: they often appear as if formed by two cells coalescing (Text-fig. 11). Occasionally they are also double the width. In the basal two-thirds the rotenoid cells are much longer than normal ones (Text-fig. 11), and they form almost a network around the outer cortex. They vary between 3 and 8 times the size of normal cells ($75 \times 20 \times 20\,\mu$ to $150 \times 30 \times 20\,\mu$ compared with $30 \times 22 \times 17\,\mu$; one $350 \times 40 \times 20\,\mu$ was seen).

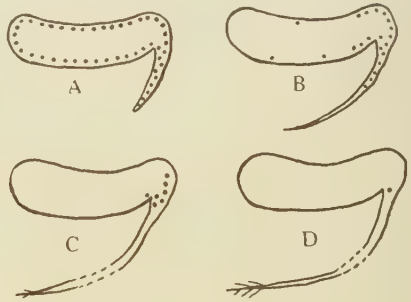
Later, as the radicle further increases in length, the rotenoids begin to disappear, from the tip first: when it is about 10 mm. long a definite reduction is noticeable; when about 15 mm. only a few rotenoid cells remain in the apical half, although many still remain in the basal half. After suberization has begun, usually when the radicle is about 20 mm. long, no rotenoid cells can be found except near the base, and these are greatly reduced in number. At this stage, rotenoids can be found only in the basal part of the cotyledons, since they have at the same time been slowly disappearing, starting at the distal ends. When the radicle is about 40 mm. long, only a few rotenoid cells can be found, at the junction of the cotyledons and the radicle: soon afterwards none can be detected anywhere in the seedling (Text-fig. 12).

There is thus a definite movement of rotenoids, first towards the newly growing tissue of the radicle, and secondly throughout the entire seedling, with their eventual complete disappearance.

Until the seedling is about six weeks old no further rotenoids can be detected in it.



Text-fig. 11.



Text-fig. 12.

Text-fig. 11. Rotenoid cells (black) in the growing radicle of *Mundulea: A*, near the tip; *B*, in the centre. Transverse.

Text-fig. 12. Distribution of rotenoid cells (black) in a germinating *Mundulea* seed. *A*, shortly after germination; *B*, radicle, 20 mm. long; *C*, radicle, 40 mm. long; *D*, radicle, 60 mm. long.

Tephrosia Vogelii.

In general, seeds of this species behave in the same way as those of *Mundulea* on germination.

The testa is discarded without apparent loss of its rotenoids: the cotyledons lose most, but not all, of theirs. During growth of the radicle rotenoid cells increase in number and occur throughout its length, most of them being somewhat larger than normal, but seldom as large as in *Mundulea*; later the number decreases, and when the radicle is about 20 mm. long its tip has lost most of its rotenoids. When it is about 60 mm. long, a few can be found at its base only: when it is larger none can be detected. The cotyledons meanwhile lose most, but not all, of their rotenoids: thus in a seedling 10 cm. long, with several rootlets, 80μ longitudinal sections showed an average of twenty-two rotenoid cells per section, compared with 114 in ripe seeds. When the cotyledons wither, rotenoid cells can still be detected in them.

Rotenoids exist in the plumule in morphologically distinct cells, as

already described on p. 657: these increase in number on the opening of the plumule: no loss has ever been observed.

The fact that rotenoids are found in the newly grown tissue means that either new supplies of them have been produced, or that they have been translocated from the cotyledons. As viable seeds of this species were plentiful, experiments were carried out to settle this point.

The radicles were dissected from 200 ripe seeds, and from 200 germinated ones with radicles about 10 mm. long, and the rotenoid contents of these two samples and of the companion cotyledons were determined colorimetrically by the methods of Gross & Smith (1934), and of Roger & Calamari (1936). In addition, in one determination, 200 seedlings with fully open cotyledons were taken as a fifth sample.

Percentage results cannot be used because of changes in weight, moisture content, etc., during growth; and therefore the weight of rotenoids per 100 seeds or seedings has been calculated for each sample. The two colorimetric methods gave different absolute results, but the ratios between them were constant, and the percentage increases of rotenoids obtained between the samples can therefore be accepted; see Table IV.

Table IV

	Ripe seed		Germinated seed		Fully open cotyledons	
	A mg.	B mg.	A mg.	B mg.	A mg.	B
Radicles	10·0	7·5	11·4	9·4	—	—
Cotyledons	15·0	10·1	18·8	11·7	—	—
Total	25·0	17·6	30·2	21·1	11·3	—
Ratio A/B	1·42		1·43		—	

Increase on germination: 20·8% by A, 19·9% by B; mean = 20·3%.

The figures under columns A are those obtained by the method of Gross & Smith, and those under B by the method of Roger & Calamari. The results show (1) an increase soon after germination of about 20% in the total amount of rotenoids, made up of increases in both the radicles and the cotyledons, and (2) a reduction, as growth of the seedling progresses, to a final amount of about 45% of the original in the ripe seed, or only 37·4% of that in the newly germinated seedling. This experiment, therefore, establishes that rotenoids are actually produced in the young germinating seed, and that shortly afterwards a large proportion of the rotenoids disappears.

In a somewhat similar experiment with *Mundulea sericea*, in which, owing to shortage of material, the radicles were not separated from the cotyledons, a total increase in rotenoids of 14·8% was obtained after germination, when the radicles were 5–10 mm. long.

Tephrosia Ehrenbergiana.

Germination phenomena in these species resemble those in *T. Vogelii*. At first, new cortical rotenoid cells are produced in the new tissue of the radicle, and can be detected there until it is about 7 mm. long (these seeds are much smaller than those of *T. Vogelii*). Thereafter, they begin to disappear from the tip upwards: when the radicle is about 15 mm. long none can be found in it. Rotenoids also diminish steadily in the cotyledons, and when these are fully open only a few rotenoid cells can be found in them: 80μ sections of one pair of such cotyledons contained an average of thirteen rotenoid cells, against sixty-seven in a ripe seed. Even when they wither the cotyledons still have a few left in them.

Tephrosia candida and *T. toxicaria.*

These species behave very similarly to the above, except that the fully open cotyledons contain only a very few rotenoid cells: in several *T. candida* cotyledons the average was one in every four 80μ sections, and in *T. toxicaria* it was slightly higher.

Tephrosia macropoda.

Only four seeds were available for germination tests, and the results are therefore meagre: they indicate that new rotenoid cells occur at first all along the growing radicle, as with the other species, and later disappear from the whole seedling except for a few in the cotyledons.

Derris uliginosa.

Germination phenomena in this species are different from those in the genera *Mundulea* and *Tephrosia*. The radicle in the seed does not contain rotenoids and the cotyledons contain them in large cavities and not in cells. On germination no rotenoids are produced in the radicle at any stage of its growth, and no new rotenoids appear to be formed in the cotyledons. As growth proceeds a small reduction of the rotenoid content of the cotyledons occurs: the original amount being reduced by 38%, as determined by colorimetric methods. The rotenoids in the plumule disappear soon after germination and none can be detected when the plumule emerges from the cotyledons.

Millettia dura.

The cotyledons, radicle and plumule of this species all contain cavities of rotenoids. On germination new rotenoid cavities are formed along the radicle, down to the growing point: they occur almost entirely in the peripheral cortex. The cavities are large enough to be readily seen along the surface of the radicle by the naked eye: they are about 350 times the size of normal surrounding cells ($270 \times 90 \times 80\,\mu$ compared with $37 \times 10 \times 15\,\mu$). As growth proceeds the rotenoids in the radicle begin to diminish from the basal end first and by the time that it is about 10 cm. long no rotenoids can be found in the upper half, the structure of which is stem, but some rotenoids still occur in the lower or root half. At this stage long cavities can be found in the cortex of the radicle: those in the upper half contain resins only, whilst some of those in the lower half also contain rotenoids. Later, no rotenoids can be detected in the root. This process is, therefore, the opposite to what occurs in *Mundulea* and *Tephrosia* spp., where rotenoids commence to diminish at the tip. The cotyledons lose rotenoids from most of the cavities around their edges but little loss occurs in the body of them: there were insufficient seeds available for quantitative work, but I doubt if the reduction in total rotenoids is large.

The rotenoids in the plumule have disappeared by the time that it emerges from the cotyledons.

Summarized table of distributions

The preceding results are summarized in Table V, which shows that rotenoids occur in many different organs: in some species, viz. *Tephrosia purpurea*, in only a few, but in others, viz. *T. Vogelii*, in many tissues. The rotenoid cells in all the stems and roots are normal ones, but in most other tissues they are morphologically distinct.

It must be understood that the volume figures are only approximate, and are based on the average of a number of measurements: in some tissues the morphologically distinct cells vary greatly in size. In the seeds of *Derris elliptica*, *D. uliginosa* and *Millettia dura* the figures are for the lysigenous cavities compared with normal cells.

Part III. Experimental

The results recorded in Part I of this paper have been entirely observational, but they have suggested many provisional hypotheses to be tested by experiment. I have carried out some preliminary work of this nature and the results are recorded below.

Table V. *Summary of plant parts containing rotenoids*

	Mundulea Sericea			*Tephrosia*																	
				Vogelii			*Ehrenbergiana*			*candida*			*toxicaria*			*macropoda*			*densiflora*			*purpurea*		
	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.
Stems	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1	–		
Roots	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1	+	N	1
Petioles	–			+	N	1	–			–			–			+	N	1	–			–		
Leaflets	–			+	D	27	–			–			–			–			–			–		
Petiolules	–			+	N	1	–			–			–			–			–			–		
Peduncles	+	N	1	+	N	1	–			–			–						–			–		
Sepals	–			+	D	19	+	D	6	–			+	D	3½				–			–		
Petals	–			+	D	27	+	D	6	–			+	D	4½				+	D	21	–		
Filaments, base	+	D	40	+	D	7	+	D	4	–			–						–			–		
stem	+	D	10	+	D	3	+	D	2½	–			–						–			–		
Anthers	+	D	80	+	D	40	+	D	34	–			+	D	10				–			–		
Ovaries	–			+	D	16	–			–			+	D	3½				–			–		
Styles	–			+	D	4	–			–			+	D	3				–			–		
Ovules	+	D	150	+	D	33	–			+	D	33	+	D	43				–			–		
Seeds: testa	+	D	16	+	D	9	–			+	NS	1½	+	D	24	+	D	29	+	D	10½	–		
cotyledons	+	NS	1½	+	NS	1½	+	D	3	+	N	1	+	NS	1½	+	N	1	+	D	1½	+	NS	1½
radicle	+	D	2½	+	D	2	+	D	2½	+	D	2½	+	NS	1½	+	D	2						
plumule	–			+	D	20	–			–			–			–								

	Derris												*Millettia*					
	uliginosa			*elliptica*			*malaccensis*			*dalbergioides*			*dura*			*usaramensis*		
	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.	Rotenoids	Type	Vol.
Stems	+	N	1	+	N	1	–			–			+	N	1			
Roots	+	N	1	+	N	1	+	N	1	–			+	N	1			
Petioles	+	N	1	–			–			–			–					
Leaflets	–			–			–			–			–					
Petiolules	–			–			–			–			–					
Peduncles	–									–			–					
Sepals	–									–			–					
Petals	–									–			–					
Filaments, base	–									–			–					
stem	–									–			–					
Anthers	–									–			–					
Ovaries	–									–			–					
Styles	–									–			–					
Ovules	–									–			–					
Seeds: testa	–			–						–			–			–		
cotyledons	+	D	43	+	D	68				+	D		+	D	27	+	D	87
radicle	–			–						–			+	D	10	+	D	
plumule	+	D	43	+	D	76				–			+	D	38	+	D	

+ = rotenoids present; – = rotenoids absent; blank = tissue not available for examination; N = normal; NS = normal but somewhat swollen; D = morphologically distinct. The columns headed "Volume" give the average ratio of rotenoid cell volume to that of the surrounding cells.

A. *Nutrition deficiency experiment*

In order to determine the effect, if any, of various mineral deficiencies on the production of rotenoids in *Derris elliptica*, I set up a pot experiment with three replicates of clonal material in each treatment. Ten-inch flower pots were used, and the plants were grown in pure sand (obtained locally and consisting of over 99% silica), and nutrient solutions applied regularly. Five treatments were given, viz. (1) Hoagland's full nutrient (see Eaton, 1936), (2) complete potash deficiency, (3) 95% deficiency of nitrogen, (4) complete calcium deficiency and (5) complete phosphorus deficiency. Tap water (which in Amani is remarkably pure and contains only 12–15 p.p.m. of dissolved salts, most of which are silica) and ordinary pure chemicals were used and, therefore, the potassium, phosphorus and calcium deficiencies, although not complete, were very nearly so.

After 18 months the three roots in each treatment, regarded as one sample, were lifted, weighed, examined microscopically and analysed. Table VI shows the results.

Table VI

	Root dry wt. g.	Ether extract dry wt. %	Rotenone dry wt. %	Rotenone per plant g.
Full nutrient	255	6·0	1·55	1·3
K deficient	215	9·4	2·55	1·8
N deficient	240	10·9	3·05	1·6
Ca deficient	120	9·6	2·60	1·0
P deficient	54	8·7	2·45	0·4

They indicate that complete deficiencies in potash and nitrogen have little effect on the yield of root, whereas calcium deficiency reduces it to half and phosphorus deficiency to nearly one-fifth. The figures for the rotenone contents of the roots are surprising: they show that full nutrient solution gave only about half as much as that given by each of the four deficiency solutions. If worked out on a basis of weight of rotenone per plant, potash and nitrogen deficiencies, especially the former, show a greater production of rotenone than full nutrient.

The experiment will be repeated on a larger scale, but the present results suggest, *inter alia*, that a poor soil may be preferable to a rich one for high rotenone content.

One interesting point was that the plants grown with a deficiency of nitrogen produced large numbers of nodules on their roots, and that many of these nodules contained rotenoid cells. *Derris* plants grown

normally have few if any nodules, and I have seldom detected rotenoids in those that do occur.

As determined by microscopic examination, the distribution of rotenoids was normal in roots of all the plants except those grown with a calcium deficiency. In these latter roots few rotenoid cells occurred in the outer xylem parenchyma which showed as a ring of non-rotenoid cells (Pl. XXXVIII, fig. 7): small roots were, however, normal. All the roots contained many starch cells.

In what was the original cutting the distribution was normal for full nutrient and potash and nitrogen deficiencies: with calcium deficiency, there were scarcely any rotenoids in the part of the cutting above ground, whilst with phosphorus deficiency there were few in the part below ground: other distributions were normal.

On the whole, it can be said that none of the deficiencies produced any great change in the rotenoid distribution in the entire plant.

B. *Light deficiency experiment*

In order to determine whether the rotenoids in *Derris* root could be used as a food reserve, a number of potted plants were placed in the dark, watered regularly, and kept there until dead. Before dying, several of them produced long etiolated shoots.

Examination under the microscope showed the rotenoids apparently undiminished in quantity, although all the starch and much of the hemicelluloses had been used up. To test this quantitatively a plot of ten 1-year-old plants in the field was covered so that light was excluded: ten plants adjacent to them in the same plot being used as controls. When dead the former were lifted, weighed and analysed. Table VII shows the results, which indicate that if any loss of rotenoids has occurred it is only a small one.

Table VII

	Dry wt. of roots g.	Rotenone %	Rotenone per plant g.
Normal plants	400	7·4	3·0
Plants kept in dark	280	8·9	2·5

As the roots of those plants kept in the dark were more breakable, a certain amount was lost in digging, and if allowance be made for this loss the value for rotenone per plant is greater than 2·5 g. It is, therefore, very improbable that the rotenoids are available as a food reserve.

C. *Experiment with* Derris *cuttings*

Cuttings from the lower, woody parts of *Derris elliptica* stems root more readily than those from the upper, green parts: the former contain rotenoids in their pith, the latter do not.

In order to test if any connexion exists between these two facts, I took green cuttings, tested them at each end to ensure that no rotenoids were present, and then provided them with rotenoids in the form of (*a*) expressed *Derris* root sap, (*b*) expressed stem sap. These saps were applied (1) by hypodermic syringe into the pith at both ends, and (2) by dipping the lower end into the sap and applying suction to the top.

Ten cuttings were used in each test. A small reduction occurred in the percentage number of cuttings which rooted and no increase in the speed of rooting was noticeable.

With root sap added, 30% of the controls rooted, while only 20% with each treatment did so: at the same time 80% of woody cuttings rooted. With stem sap, the corresponding figures were 60, 50, 50 and 80%.

It appears, therefore, that the rotenoids in the lower end of *Derris* stems have no effect on their rooting qualities.

Discussion

This exploratory study has provided information on rotenoid distribution in several species of plants, and has suggested lines for future investigation. Although some of the facts relate to certain species only, a number of generalizations appear to be justifiable.

In every species that contains rotenoids in any of its tissues there are rotenoids in the seeds and, with one exception, in the roots: the exception is *Derris dalbergioides*, the seeds of which contain such small traces of rotenoids that smaller traces in the roots may well have been overlooked altogether.

The species in which rotenoids have been detected show a range of rotenoid content from the mere traces in the seeds of *D. dalbergioides* to the appreciable quantities in almost every organ of *Tephrosia Vogelii*.

In six species grown from seed, rotenoids were first detected in the young roots, in the cortex opposite the protoxylem and primary medullary rays; thereafter spreading, to a greater or lesser extent, around the cortex and through the xylem parenchyma. In young roots of older plants the process is similar. There must be some significance in the fact

that rotenoids are so frequently laid down in the cortex opposite the protoxylem, but I am unable to suggest what it may be. The suggestion that it is only a question of waste products moving along the rays into what will eventually be the bark cannot hold, because the rotenoids soon spread to other tissues.

Of the species examined fully, four have rotenoids in their stamens, and four (not all the same four) in their ovules, all in morphologically distinct cells. Rotenoid cells in young ovules have, in every instance, persisted in the testa of the ripe seed. This appears to be the rule, so that, for example, with the knowledge that the testa of *T. macropoda* contains rotenoids, I confidently expect to find them in young ovules when flower buds become available.

I consider it possible that some chemical relationship exists between the rotenoids and the resins in these plants, for they generally occur together in the same cells. This is particularly true for mature *Derris elliptica* roots, where all resin cells in the xylem parenchyma contain rotenoids. In very young anthers and ovules of *Mundulea sericea* the resin cells contain no rotenoids, but, as the organs grow, first one or two and then gradually all of the resin globules in a cell are found to contain rotenoids. This indicates that the resins and rotenoids are intimately mixed in each cell. The pith of *Derris elliptica* stems contains many resin cells, and in the lower woody parts many also contain rotenoids: as the stem grows and the woody tissue extends upwards, more of the resin cells become rotenoid-containing. In *D. uliginosa* many of the resin cells all the way up contain rotenoids, irrespective of the woodiness of the stem. In some species the anthers and certain other tissues do not contain rotenoids, but resin cells are found that are similar in appearance to, and in the same positions as, rotenoid cells in other species.

In considering the relationship between the resins and the rotenoids it may be recalled that many resins on melting with potash yield resorcinol, and that an alkyl resorcinol has recently been obtained from the tubaic acid fraction of rotenone.

As far as I am aware no suggestions have been put forward by other workers as to the physiological role that rotenoids may play in plants. Two hypotheses at once present themselves, (1) that rotenoids play an active part in plant metabolism, e.g. as food reserves; (2) that they are waste products. My results so far suggest a third hypothesis, namely, that rotenoids play an active part in the germinating seed but are waste products in all other tissues.

On germination, *Mundulea sericea* and the five *Tephrosia* species of

which viable seeds were available behave very similarly: rotenoids increase in both radicle and cotyledons and then diminish, in the former to nil, in the latter to a varying degree, the extremes being by 55% in *T. Vogelii* and by 100% in *Mundulea sericea*.

In *Millettia dura* the process is the same except for a smaller reduction in the cotyledons. In *Derris uliginosa* no initial increase occurs and the final reduction in the cotyledons is by 38%. The rotenoids in the plumules of these two species, however, disappear entirely. This disappearance of rotenoids during germination is consistent with the third hypothesis.

In all other tissues, including the testa, rotenoids appear to be waste products, in the sense that they are not available as food reserves, since all these tissues can wither and die without any apparent loss of rotenoids, and this is so whether the rotenoid cells are normal or morphologically distinct.

Amani strain *D. elliptica* has been grown under varying conditions of rainfall, altitude, soil, etc., i.e. with about 100 in. of rain in the hills at 3000 ft. altitude, with about 50 in. at a few hundred feet elevation, under irrigation on the plains, and in sandy as well as very heavy soil. In every case the percentage rotenone content increased steadily, to a maximum, with the growth of the root.

This indicates that rotenone production in the root is a regular and constant process, as would be expected if rotenoids were waste products steadily accumulating during plant growth. Experiments have shown that the rotenoids are not reduced in amount by conditions that reduce food reserves, and this favours the hypothesis that they are waste products, not utilizable as food.

The increase in the number of rotenoid cells, especially in the pith, at nodes and to a lesser extent at root branchings, suggests that rotenoids may here be a by-product of secondary or lateral growth. Similar accumulations of rotenoids also often occur at other junctions, e.g. of the peduncle with the stem and with the flower, of the filament with the anther and with the receptacle, and of the funicle with the integument. I have also found large amounts of peroxidases at many of these junctions, especially at the nodes. Other workers have shown that accumulations of other substances, e.g. iron, occur at nodes. Nodes are in general points of active growth, and the presence of increased amounts of rotenoids here lends support to the idea that they are in some way directly connected with the growth of the plant; but as they remain undiminished after secondary or lateral growth has ceased, their presence cannot be

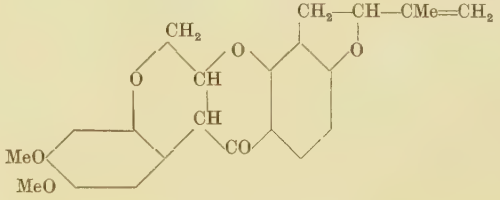
accounted for by the assumption that they are substances of use to the plant as a food supply.

Except in germinating seeds, only two instances are known of proven lability of rotenoids, i.e. their disappearance from the pith of *Tephrosia Vogelii* and *Mundulea sericea*, especially at the nodes. Rotenoids occur in the non-lignified pith but cannot be detected after lignification. I have no evidence to show whether these rotenoids have been translocated prior to lignification or destroyed during that process.

On the other hand I have detected rotenoids in tissues usually connected with translocation, e.g. sieve tubes and medullary rays: I am of the opinion, however, that rotenoids are not normally translocated as such. For instance, *Derris elliptica* plants grown from cuttings with no original rotenoids develop them only in the roots until the plants are fairly mature, and they must therefore have passed down the stem in some non-rotenoidal form and been converted to rotenoids in the root.

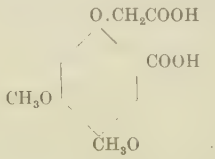
With *D. elliptica*, the root as it grows steadily accumulates rotenoids and starch until all the xylem parenchyma cells contain either the one or the other: except for their contents these cells appear identical. It is, therefore, probable that both starch and some form of rotenoid precursor are passing down continuously from the leaves to the root, where in some cells starch is stored and in others the rotenoid precursors, which are then converted to rotenoids *in situ*. Here the rotenoids appear to be waste products and, therefore, it may be assumed that the precursors have performed some useful function before being converted to waste products. It is also conceivable that simple substances may pass down from the leaves into the roots or other tissues, and then undergo a complex synthesis *in situ*: this, I consider, less likely.

It is very difficult to suggest in what way the rotenoids can be utilized in the plants. Rotenone, $C_{23}H_{22}O_6$, which is usually the chief toxic constituent to be isolated from these plants, has been assigned the structural formula



and chemically the other toxic constituents, tephrosin, deguelin and toxicarol, are closely allied: the first three all give derric acid, $C_{12}H_{14}O_7$,

on oxidation. Rotenone is considered to consist of a rissic acid fraction, $C_{11}H_{12}O_3$, containing two methoxy groups, combined by a carbonyl group to a tubaic acid fraction, $C_{11}H_{10}O_2$, thus $C_9H_6O(OCH_3)_2.CO.C_{11}H_{10}O_2$. On oxidation *in vitro* several of the rotenoids yield rissic acid, $C_{11}H_{12}O_7$, with the structure



Whilst it is more likely that these less complex substances could be utilized by the plant, it appears unlikely that such complex substances as rotenoids would be synthesized merely to be utilized for producing the simpler ones. It seems reasonable to assume that the synthesis and degradation of rotenoids are in themselves of some importance in the plant metabolism. It may be that they play a role somewhat similar to that of the respiration chromogens, substances which unite readily with free oxygen under the influence of oxidizing enzymes to produce water and respiration pigments. These pigments are capable of acting as acceptors of hydrogen, to reproduce the original chromogens and oxygen. With gentle oxidation rotenone and deguelin each lose two hydrogen atoms to give dehydrorotenone and dehydrodeguelin: on reduction with hydrogen they add on two hydrogen atoms to give dihydrorotenone and dihydrodeguelin. Neither reaction appears to be reversible *in vitro*, but it is possible under the influence of an enzyme that they act in the plant as hydrogen acceptors, or play some similar simple role. Clark & Keenan (1933) have isolated dehydrodeguelin and dehydrotoxicarol from a *Derris* root which contained no rotenone.

As the rotenoids usually occur mixed with fixed oils in the cotyledons, it is possible that they may take some part in the hydrolysis of these oils during germination. Preliminary experiments were, however, inconclusive.

Work now in progress with interspecific grafts of *Derris* may throw light on the mechanism of rotenoid formation. Thus *D. polystachya*, which contains no rotenoids, has been grafted on to *D. elliptica*, and vice versa. A preliminary result from *D. elliptica* on *D. malaccensis* tends to show that the root of the latter accumulates abnormal amounts of rotenoids as a result of the influence of the scion.

While the evidence suggests that rotenoids perform some useful function in the germinating seeds and young seedlings of these plants,

but may be waste products in all other tissues and are certainly not utilizable as a reserve food supply, it is admittedly exceptional for the same substance to function as a physiologically useful compound and as a waste product, especially in the same plant. The question, therefore, arises whether the rotenoids in the seeds are indeed identical with those in the other tissues. On this point I have some evidence for *Mundulea sericea*. Rotenone is the chief rotenoid extracted from both the stem and the seed and, therefore, these organs contain either rotenone or a rotenone precursor that changes to rotenone during the process of extraction. Since all rotenoids disappear from the seed soon after germination, either rotenone can act as a useful compound in the seed and a waste product in the stem, or one of them, e.g. the seed, contains a rotenone precursor capable of changing to a non-rotenoid during germination, whilst the stem contains rotenone itself or a precursor not normally capable of such a change.

Finally, there is the possibility that other toxic substances that do not respond to Durham's test may occur in some of the tissues, but at present it is not possible to identify any. There is no evidence, however, that any appreciable quantities of such substances occur.

In conclusion, it is an interesting fact that rotenoids are confined to a narrow range of plants: apparently to the two subtribes Tephroseae and Lonchocarpeae, of the different tribes Galageae and Dalbergeae, respectively. As far as I am aware, only species of the genera *Mundulea*, *Tephrosia*, *Millettia*, *Derris* and *Lonchocarpus* contain rotenoids, and not all species of any of these genera contain them. About fifty species are said to be toxic to insects or fish, but the number of rotenoid-containing species is probably higher than this, since some reputed non-toxic ones contain small amounts of rotenoids (e.g. *Tephrosia densiflora*) and many have not yet been examined. The taxonomic distribution of these species within the two subtribes appears to be random, but the general uniformity in the behaviour of rotenoids during germination may be evidence of a single evolutionary origin.

Summary

1. All available parts at all available stages of growth of twenty-two species of Papilionaceae have been examined microscopically for the presence of rotenoids: fourteen contain them, and Table V summarizes all the plant parts in which they occur.

2. The rotenoid content varies from mere traces in the seeds only of

one species to appreciable quantities in nearly every organ of another species.

3. Seeds of twelve of the rotenoid-containing species were available: all contained rotenoids. It appears to be the rule that if any species contains rotenoids in any tissue it will contain them in its seeds.

4. Roots of thirteen of the species were available: twelve contained rotenoids.

5. In general, rotenoid cells in the roots and stems are normal ones, but in all other tissues they are usually morphologically distinct, and frequently relatively very large.

6. Rotenoids occur in the seeds of species of *Millettia* and *Derris* not in cells but in lysigenous cavities.

7. In many species rotenoids are first detected in the cortex of the root opposite the protoxylem and primary medullary rays.

8. In general, the roots contain rotenoids in the xylem parenchyma, especially in the medullary rays, and in the cortex. In stems they occur mainly in the pith, when not lignified, and also in the phloem, especially in the rays, and in the cortex.

9. At nodes there is frequently an increase in the number of rotenoid cells, especially in the pith.

10. On germination, the seeds of *Mundulea* and *Tephrosia* species at first produce new rotenoid cells, accompanied by an increase in total rotenoid content, in the growing radicle and in the cotyledons. Later, the rotenoids diminish to nil in the radicle and to between 45 % and nil, for different species, in the cotyledons. With *Millettia*, new rotenoid cavities are at first formed in the radicle, and later all rotenoids disappear from it: only a small reduction in the rotenoid content of the cotyledons, however, occurs. With *Derris*, no new rotenoids are formed in the radicle: the amount in the cotyledons in one species diminished to 62 %.

11. Rotenoids in the plumules of *Millettia* and *Derris* species disappear soon after germination.

12. Two instances only of lability of rotenoids have been proved: in the germinating seeds of all species, and in the lignifying pith of the stems of two species.

13. *Derris elliptica* plants placed in the dark until dead show no reduction in the amount of rotenone per plant: indicating that the rotenone is not available as a food reserve.

14. The application of the sap from *Derris* roots and stems to *Derris* cuttings produces no increase in percentage rooting or in the speed of rooting.

15. The results suggest that rotenoids are of some direct use to the germinating seed and seedling, but that they are probably waste products in all other tissues.

Finally, I wish to thank F. J. Nutman for the encouragement and help that he has given me during this investigation and the writing of the paper. I wish also to thank P. J. Greenway for the collecting of some and the naming of much of the plant material used, and H. H. Storey and R. E. Moreau for suggestions and criticism in the writing of this paper, especially the discussion.

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EXPLANATION OF PLATES XXXV–XXXVIII

PLATE XXXV

Fig. 1. 80 μ transverse section of stem bark of *Mundulea sericea*, showing rotenoid cells, dark, in medullary rays. 25 ×.

Fig. 2. 80 μ longitudinal section of stem pith of *Derris elliptica*; rotenoid cells, dark, are seen in chains. 20 ×.

Fig. 3. 80 μ transverse section of root bark of *Mundulea sericea*: rotenoid cells dark. 25 ×.

Fig. 4. 80 μ longitudinal section of bark of young root of *Mundulea sericea*, showing globular nature of contents of rotenoid cell. 125 ×.

Fig. 5. 80 μ transverse section of young root of *Tephrosia Vogelii*, showing rotenoids, black, in the cortex opposite the primary medullary rays, and also in the rays. 37 ×.

Fig. 6. 80 μ transverse section of mid-rib of leaflet of *Tephrosia Vogelii*, showing rotenoid cells, black, in the collenchyma. 20 ×.

Fig. 7. 6 μ transverse section of *Tephrosia Vogelii* leaflet, showing three large rotenoid cells, white. 125 ×.

Fig. 8. 6 μ transverse section of *Tephrosia Vogelii* petal, showing two large rotenoid cells, white. 125 ×.

PLATE XXXVI

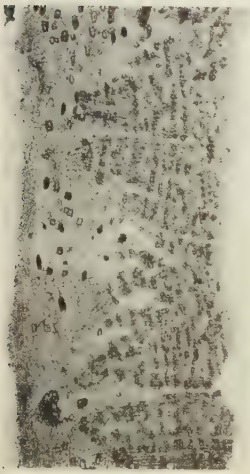
Fig. 1. 6 μ transverse section of *Mundulea sericea* filament stem, showing one large rotenoid cell containing globules. 150 ×.

Fig. 2. 6 μ transverse section of *Mundulea sericea* filament base, showing one large rotenoid cell containing seven globules. 150 ×.

Fig. 3. 6 μ transverse section of *Mundulea sericea* anther, showing two large rotenoid cells. 275 ×.

Fig. 4. 6 μ transverse section of *Tephrosia Vogelii* anther, showing six large rotenoid cells in the connective tissue. 110 ×.

Fig. 5. 80 μ longitudinal section of very small ovule from *Mundulea sericea* bud, showing three rotenoid cells, black. 160 ×.



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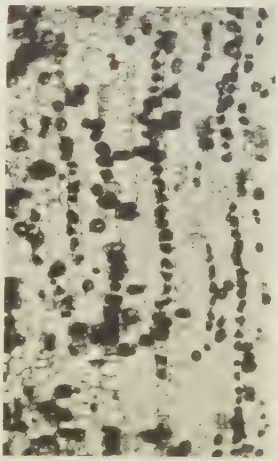

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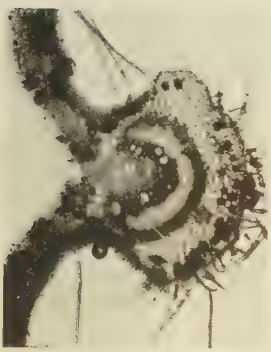

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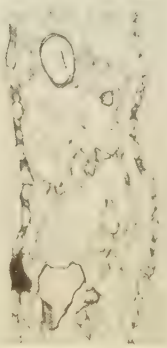

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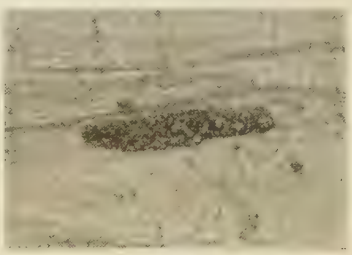

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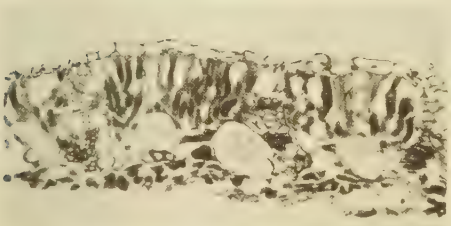

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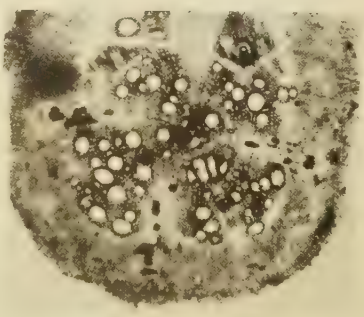

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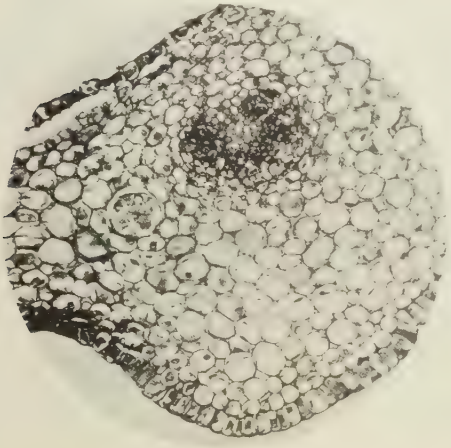

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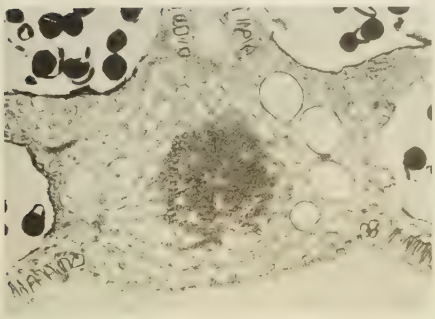

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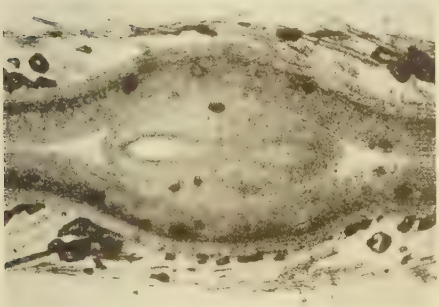

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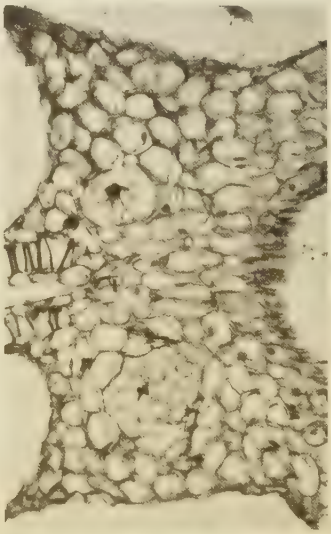

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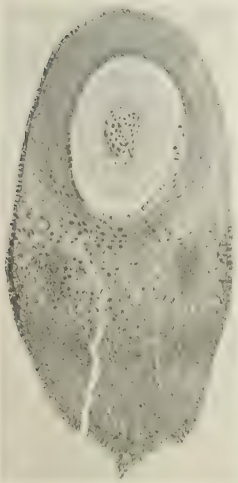

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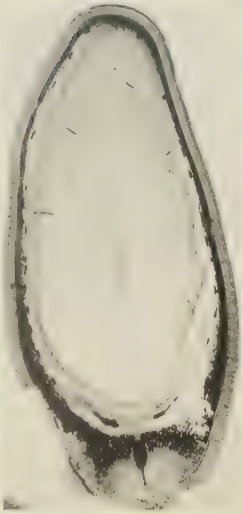

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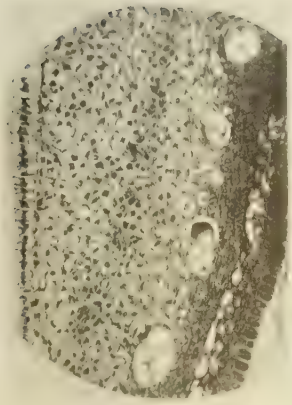

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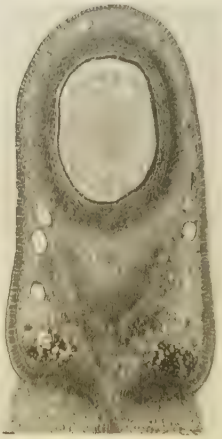

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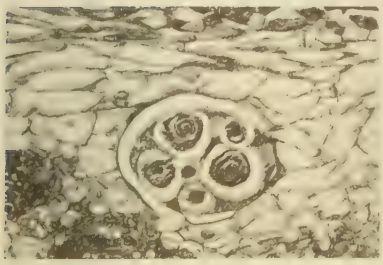

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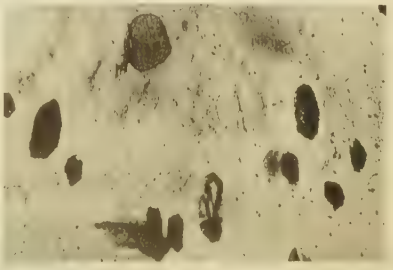

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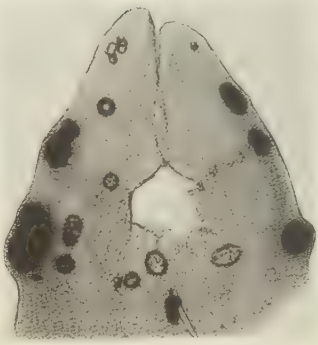

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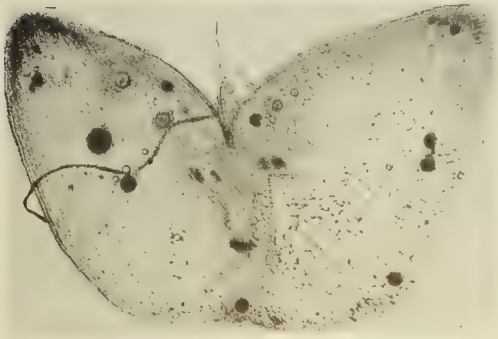

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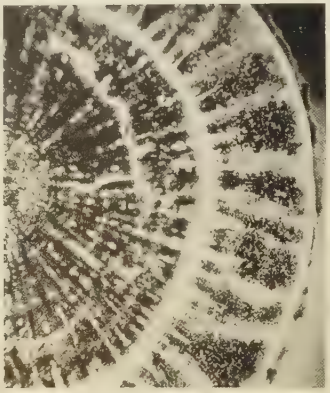

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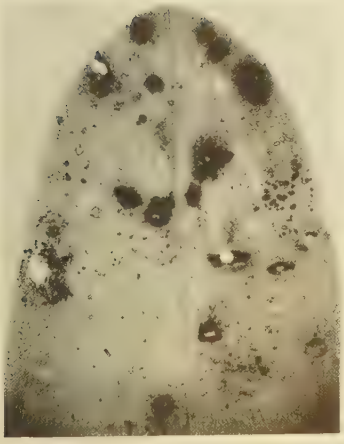

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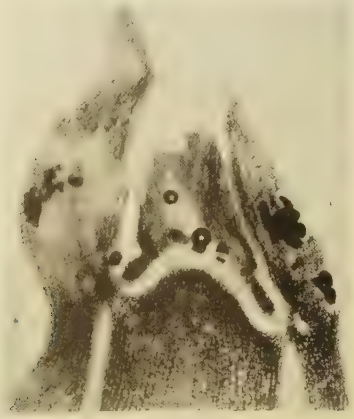

Fig. 4.

PLATE XXXVII

Fig. 1. 6 μ transverse section of small ovule of *Mundulea sericea*, showing two rotenoid cells. 50 ×.

Fig. 2. 80 μ transverse section of somewhat larger ovule of *Mundulea sericea*, showing rotenoid cells spreading around it. 150 ×.

Fig. 3. 6 μ longitudinal section of part of fairly large ovule of *Mundulea sericea*: note the globular contents of the four rotenoid cells. 150 ×.

Fig. 4. 80 μ transverse section of immature *Mundulea sericea* seed: cotyledons not yet completely differentiated. Rotenoid cells, black, occur only in the testa. 20 ×.

Fig. 5. 80 μ transverse section of ripe seed of *Mundulea sericea*. Rotenoid cells are black. 25 ×.

Fig. 6. 6 μ transverse section of small ovule of *Tephrosia Vogelii*. Large white cells are rotenoid ones. 35 ×.

Fig. 7. 6 μ transverse section of a large rotenoid cell, with globular contents, in *Tephrosia Vogelii* ovule. 200 ×.

Fig. 8. The parenchyma layer of the seed testa of *Tephrosia Vogelii*: rotenoid cells black. 100 ×.

PLATE XXXVIII

Fig. 1. 80 μ transverse section of *Tephrosia Vogelii* ovary: small black cells in the parenchyma of the walls are rotenoid ones. 12 ×.

Fig. 2. 80 μ transverse section of *Tephrosia Vogelii* style, showing rotenoid cells, black, around the periphery. 125 ×.

Fig. 3. 80 μ transverse section of part of *Derris elliptica* seed, showing rotenoid cavities. 17 ×.

Fig. 4. 80 μ longitudinal section of plumule of *Derris uliginosa*, showing rotenoid cavities in the abaxial sides of the immature leaflets. 35 ×.

Fig. 5. *Millettia dura* seed with testa removed, showing rotenoid cavities, white, near the surface. 3 ×.

Fig. 6. 80 μ longitudinal section of plumule of *Millettia dura*, showing rotenoid cavities, black. Note the epidermal expansion opposite the cavities. 45 ×.

Fig. 7. 80 μ transverse section of *Derris elliptica* root grown with calcium deficiency, showing a ring of non-rotenoid cells. 12 ×.

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STUDIES UPON THE TIME OF FLOWERING OF PLANTS

ANATOMICAL, FLORISTIC AND PHENOLOGICAL ASPECTS OF THE PROBLEM

By JOHN GRAINGER

Tolson Memorial Museum, Ravensknowle, Huddersfield

(With 9 Text-figures)

CONTENTS

Introduction

Much botanical research of recent years has centred round the causes and control of flowering. Manipulation of temperature is effective for the early blooming of bulbs; variations in photoperiod affect certain other species, whilst many kinds are amenable to vernalization (Whyte & Hudson, 1933). The attainment of a suitable degree of preponderance of carbohydrate over nitrogen in metabolism has been advanced as a physiological cause of flowering. No one of these causes appears adequate to explain the flowering of all kinds of plants, and it becomes increasingly evident that the causes are interrelated. Roberts & Struckmeyer (1938) demonstrated that photoperiodic response in many plants is dependent upon temperature. Grainger (1938) showed that the relative carbohydrate metabolism of late-flowering chrysanthemums is dependent upon photoperiod, though Sheard (1938), working with similar material, found no evidence that it was the carbohydrate-nitrogen ratio in the growing point which was affected.

Any attempt to elucidate the action of various factors upon the time of flowering would, moreover, seem to demand a relation to the time when the flower bud was first formed, and not only to the time when it emerged. It is known that many plants make their flower buds a considerable time before the emergence of bloom. Thus apples (Bijhouwer, 1924; Gibbs & Swarbrick, 1930), pears (Luyten & de Vries, 1926), cherries (Versluys, 1921) and plums (Luyten, 1921) all make flower initials in July or August, but are not covered with blossom until the following May. Lilac, azalea and rhododendron (Luyten & Versluys, 1921) and lily-of-the-valley (Zweede, 1930) make their flower initials in June or July for the following spring. Hyacinth (Blaauw, 1920), tulip Mulder & Luyten, 1928) and daffodil (Huisman & Hartsema, 1933; Grainger, 1935) make flower buds during the summer months, to emerge naturally in the succeeding April, or to be forced into maturity at Christmas. Most of the data which is available refers to trees or to bulbs, which have a specialized organization, and information as to the times when biennials, herbaceous perennials and shrubs make their flower initials is meagre.

The absence of such data militates against a true valuation of any possible photoperiodic response in many plants. Thus the daffodil is a short-day plant when considered from the standpoint of its flower emergence, but a pronounced long-day species if the time of formation of its flower initial be the criterion. It is, moreover, necessary to know more about the times of flower-bud formation before the effects of climate can be evaluated correctly. The present paper gives the results of an investigation into the times of formation of the flower initial in a number of plants of diverse habit, and considers the general effects upon the time of flowering, of such factors as climate and plant form. Further studies upon the relative carbohydrate metabolism of several plants in relation to their organization for flowering will be published later.

THE ANATOMICAL ORGANIZATION FOR FLOWERING

Methods

The general method was to examine at intervals a number of buds or growing points which, by their size, or their position upon the plant, might be presumed suitable for the formation of flower initials. Such buds were dissected separately, and examined under suitable magnification of from 10 to 40 diameters. This was usually sufficient to reveal the nature of the growing point with certainty. A method of retrospective checking was adopted when a considerable number of plants of uniform growth was available. Average counts were made of the number of leaf initials nearer

to the growing point than the smallest expanded leaf. Such leaves were marked in a number of plants which were not dissected until the next examination, when counts were made from the marked leaves. When the flower initial was readily visible it was possible to refer its formation to a time between two previous dissections.

It was sometimes difficult to obtain floriferous plants. Certain patches of wood anemone and blackthorn were marked as floriferous in 1937, but material from them showed no flower buds, nor did they flower, in 1938. It was therefore the practice to examine material from more than one station, as a check, though results given in Tables I–IV are stated for only one particular locality for each species. Variation in the anatomical phenology in different localities in the neighbourhood of Huddersfield was frequently as much as a fortnight, and occasionally slightly more.

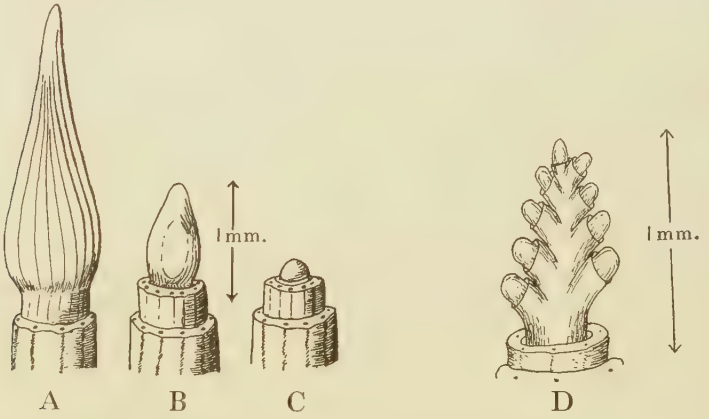


Fig. 1. *Holcus mollis*. A, B and C, dissections of the vegetative growing point, 3 Apr. 1938. D, panicle initial, 24 Apr. 1938.

Results

Dissections of more than a hundred species growing in the vicinity of Huddersfield were made in 1937, 1938 and in the early months of 1939. Some of the most complete results are shown by Tables I–IV, and some of the dissections are further shown by Figs. 1–7. It seemed possible to recognize several types of organization for flowering, and for convenience of reference they are described below.

Classification of types of organization for flowering

A. *Direct flowering plants.*

Development from the first appearance of the flower initial to the open bloom is uninterrupted.

(i) *Direct flowering from the period of maximum vegetative growth.* Flowers are initiated when leaf growth is at a maximum, and develop

into the complete bloom without interruption. This is the commonest type of floral organization, and results for twenty-eight representative species are given in Table I. Figs. 1–3 further elucidate the transition from vegetative to flower growing points. *Holcus mollis* (Fig. 1) has a terminal flower; *Epilobium angustifolium* (Fig. 2) bears axillary leaf buds at first, but later flowers are produced in the axils of younger leaves; and *Dianthus Allwoodii* (Fig. 3) begins to form its terminal flowers in spring. Certain peculiarities of its growth are discussed later in this paper.

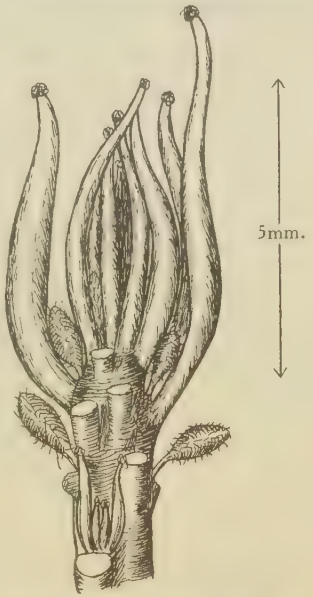


Fig. 2. *Epilobium angustifolium*. Transition from vegetative buds to flower buds in the axils of leaves, 1 June 1938. The shoot was 2 ft. above ground.

(ii) *Direct flowering from the period of minimum vegetative growth.* Flowers are initiated in late autumn or winter when leaf growth does not take place or is at a minimum. Development into the complete bloom is not interrupted, and this group includes many plants which flower early in the year. Representative species are shown in Table II, whilst Fig. 4 (*Saxifraga tridactylites*) and Fig. 5 (coltsfoot) show actual dissections. *S. tridactylites* is an evergreen, but coltsfoot makes and develops its flowers entirely at the expense of stored food, when no vegetative development of the plant takes place.

B. *Indirect flowering plants.*

A period of rest intervenes between the formation of the flower bud and its emergence into bloom.

(i) *Indirect flowering from the period of maximum vegetative growth.* Flowers are initiated towards the end of summer, and usually every organ of the future flower is complete in miniature before the leaves fall. Flowers usually emerge in the early months of the succeeding year; most fruit trees belong to this category. Table III shows that several herbaceous perennials, in addition to trees, also adopt this organization for flowering. Fig. 6 illustrates the flower bud of the bilberry, *Vaccinium*

myrtillus, which is notable amongst the species studied for the long period during which the flower bud remains dormant. It can be dissected in a fully formed condition on 17 July, and does not flower until the following

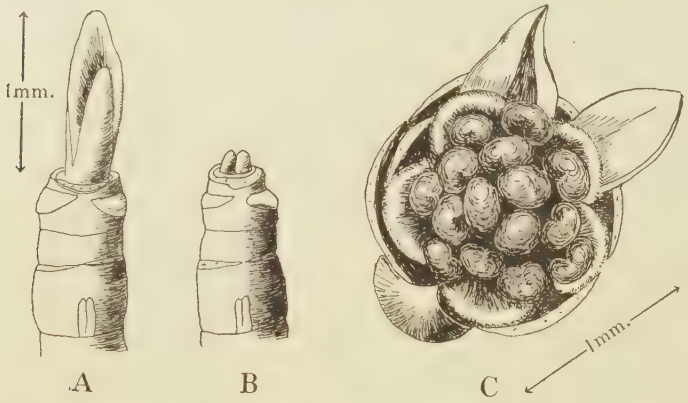


Fig. 3. *Dianthus Allwoodii*. A and B, vegetative growing point, 19 Mar. 1938. C, plan view of flower initial, 17 May 1938.

May. Flower formation probably begins early in June, so that the floral bud takes about 11 months from initiation to emergence. Most fruit trees have a corresponding period of approximately 9 months.

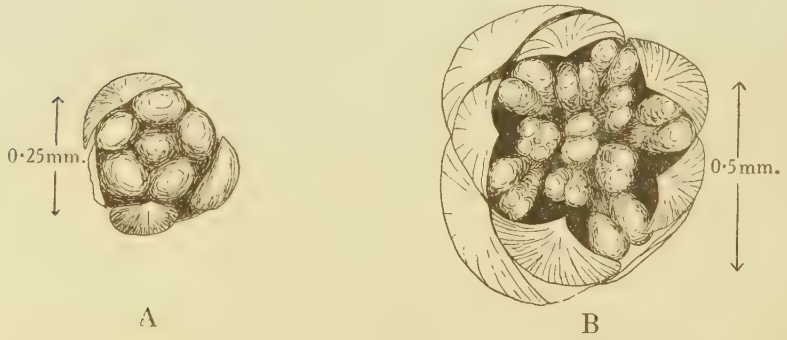
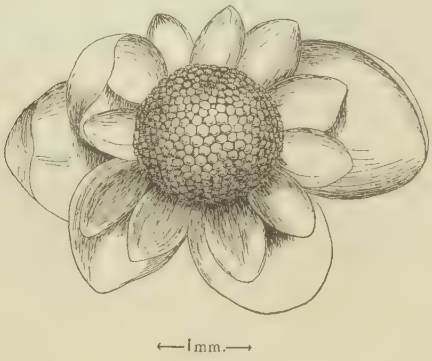


Fig. 4. *Saxifraga tridactylites* Hort. A, plan view of growing point, first initiation of flower, 29 Oct. 1938. B, plan view of growing point with flower initial, 14 Dec. 1938.

(ii) *Indirect flowering from the period of minimum vegetative growth.* Flowers are initiated after the leaves have withered, and after every organ of the future flower is complete in miniature, a period of dormancy

follows. Flowers usually emerge in late winter or early spring, along with the leaves. This type of organization has not been studied in the present investigation, but work upon the bulbs of horticultural value, already cited, have shed much detailed light upon this class.



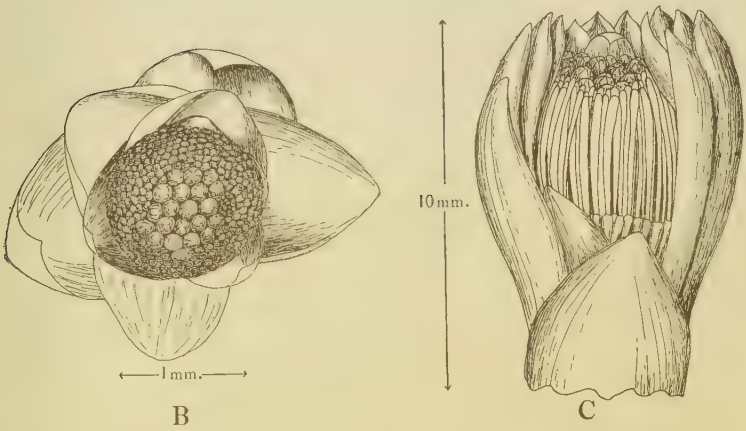


Fig. 5. Coltsfoot, *Tussilago farfara*. A, plan view of the inflorescence initial, 17 Sept. 1938. B, plan view of inflorescence initial, 29 Oct. 1938: 21 disk florets developing. C, side view of inflorescence 3 Dec. 1938. The total length of the inflorescence, shown as 10 mm. on 3 Dec., had increased to an average of 17 mm. on 1 Jan. 1939, when the florets also appeared yellow.

C. *Cumulative flowering.*

Flower initials are produced in regular succession, and all emerge together. Tables IV and V, and Fig. 7 illustrate this behaviour. The plantains and dandelion are all examples of long-term preparation for a period of maximum flowering. Flowering initials are formed in the axils of the rosette leaves, beginning about November for the dandelion and *Plantago lanceolata*, and in early spring for *P. major* and *P. media*. The

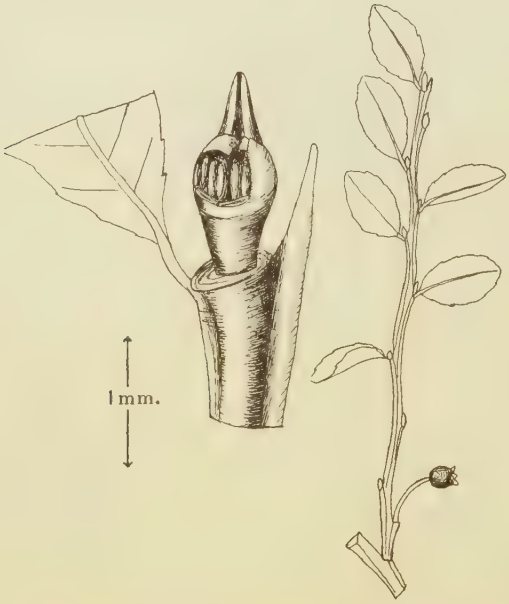


Fig. 6. Bilberry, *Vaccinium myrtillus*. Dissection of flower initial from terminal bud of the adjoining shoot, 17 July 1938.

inflorescences all emerge together at the respective times shown in Table IV for each species. Table V and Fig. 7 show the relative sizes of inflorescence initials in *P. major* on 22 May 1938; they all emerged together about 25 June. Vegetative buds appear in the axils of a few leaves, and proliferate to form the clumps characteristic of these plants. *P. lanceolata* and dandelion make their inflorescence buds largely during the period of presumed minimal photosynthesis, whilst *P. major* and *P. media* are florally determined when leaf growth is vigorous, thus suggesting the same subdivisions as are mentioned above for direct- and indirect-flowering plants.

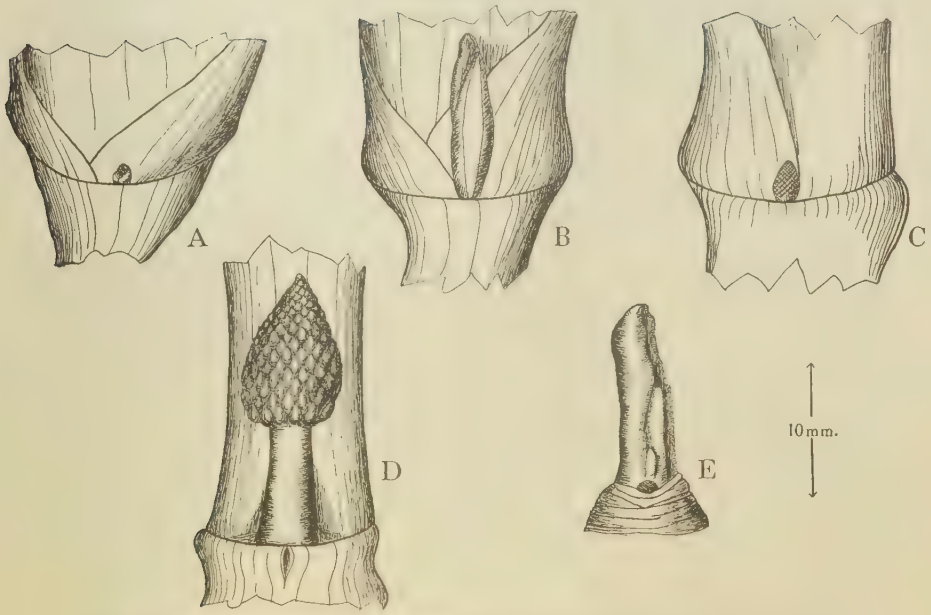


Fig. 7. Broad-leaved plantain. *Plantago major*. Leaf buds and inflorescence initials referred to in Table V. The base of the subtending leaf is shown pressed away from the main shoot in each case. A, outermost vegetative bud. B, innermost vegetative bud. C, outermost inflorescence bud. D, largest inflorescence bud. E, innermost inflorescence bud.

D. *Climax flowering.*

The plant is entirely vegetative for a number of years, and then entirely floriferous for a year, e.g. *Amorphophallus titanum* in the Hamburg Botanical Garden. This species is vegetative for about 40 years, and then sends forth a flower. Some species of agave behave similarly.

The chief significance of these results lies in Tables II and IV. Many plants have been found (Table II) which begin to make their flower initials when vegetative growth is at a minimum (e.g. saxifrage) or when there is no leaf growth (e.g. coltsfoot). It would appear difficult to align this behaviour, and that of § B (ii) above (bulbs), totally with any external cause. Cumulative flowering (Table IV) is probably a direct response to temperature. Preliminary experiments with *Plantago lanceolata* suggest that the inflorescence buds can be made to emerge in

March if the temperature is raised artificially to the average of May, when flowering usually takes place. This further suggests that flower initiation and emergence are separately favoured by different external conditions.

Table I. *Direct flowering from the period of maximum vegetative growth. Times of flower bud formation and of flowering*

Name of species	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Aira flexuosa L.	.	.	.	− 3	− 6 +12	*30	.	.	.	.	.	.
Angelica sylvestris L.	.	.	−17	−24	−15	+ 6	+	*4	.	.	−12	.
Calceolaria polyrhiza Hort.	.	.	.	− 3	+16	*20	.	.	.	.	.	.
Calluna vulgaris Salisb.	.	.	.	−10	−14	+30	+	*20	.	.	.	.
Campanula rotundifolia L.	.	.	− 3	−24	.	−11	+ 6 *24	.	.	.	.	.
Carduus lanceolatus L.	.	.	.	− 3	−16	+ 6	*15	.	.	.	.	.
C. palustris L.	.	.	.	−24	−14	+ 6	*15	.	.	.	.	.
Centaurea nigra L.	.	.	.	− 9	−20	+ 6	+	*13	.	.	.	.
Convolvulus sepium L.	.	.	.	−24	+15	*25	.	.	.	.	.	.
Digitalis purpurea L.	−8	.	− 3	+ 3	+ 7	*25	.	.	.	.	.	.
Epilobium angustifolium L.	.	.	−31	−17	− 7	+ 1	*4	.	.	.	.	.
Erica cinerea L.	.	.	.	−10	.	−12	+26	*20	.	.	.	.
Hedera helix L.	.	.	.	.	−11	− 6	+15	+	*20	.	.	.
Heracleum sphondylium L.	.	.	.	−24	+ 7	+	*6	.	.	.	.	.
Hieracium pilosella L.	.	.	.	− 6	.	.	*2	.	.	.	.	.
H. boreale Fr.	.	.	−31	.	.	.	*4	.	.	.	.	.
Holcus mollis L.	−2	.	.	− 3 +24	+	+	*6	.	.	.	.	.
Mentha sativa Hort.	.	.	.	.	−10	+25	+	*20	.	.	.	.
Rosa canina L.	.	.	−12	+24	+ 7	*25	.	.	.	.	.	.
Rumex obtusifolius L.	.	.	− 2	−24	+ 1	*25	.	.	.	.	.	.
Scabiosa succisa L.	.	.	.	− 9	−28	+22	+	*13	.	.	.	.
Sedum acre L.	.	.	.	− 3	+20	*30	.	.	.	.	.	.
S. anglicum Huds.	.	.	.	− 3	+20	+	*17	.	.	.	.	.
S. spectabile Hort.	.	.	.	− 3	+20	+	*10	.	.	.	−16	−14
Stachys betonica Benth.	.	.	.	− 9	−17	.	.	*12	.	.	.	.
Teucrium scorodonia L.	.	.	.	−10	−17	.	*15	.	.	.	.	.
Urtica dioica L.	.	.	−16	+ 9	+28	+	*12	.	.	.	.	.

The numbers represent the days of the months under which they occur. + and − refer to flower initials, and * denotes the time of first flowering or maximum flowering. Thus " − 17" under the month of April means that no flower initial could be found on 17 April; " +15" under July indicates that flower initials were observed on 15 July, and "*20" under August means that the species was first observed to flower on 20 August.

Each result is the product of a number of dissections, usually not less than six, and if one dissection yields a flower bud, the result is shown as + in the table. Plus signs without numbers are included with some species to indicate continuity. Thus in *Hedera helix* flower initials which can be found on 15 July would ultimately form the complete flowers which emerge on 20 September.

Abnormal flowering in autumn

The very mild autumn of 1938 provided opportunity for the observation of numerous plants which flowered out of their usual season. Table VI enumerates twenty-eight typical species which were observed personally or were reported in response to an appeal for information in

Table II. *Direct flowering from the period of minimum vegetative growth. Times of flower bud formation and of flowering*

Name of species	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Anthriscus sylvestris Hoffm.	−10	+25	+31	+	*20	.	.	.	.	.	.	.
Chrysanthemum leucanthemum L.	.	−26	+12	+16	+	*15	.	.	.	.	.	.
Dactylis glomerata L.	−10	+26	+	+10	+	*10	.	.	.	−29	.	− 3
Dianthus Allwoodii Hort.	−17	− 8	−19	+ 1	+17	*16	.	.	.	.	−16	− 3
Lonicera peri-clymenum L.	− 6	.	+12	+ 9	+	+	+	*10	.	.	.	−10
Lupinus polyphyllus Hort.	+12	+20	+	+ 9	*22	.	.	.	.	.	− 5	+11
Lychnis dioica L.	− 1	+26	+ 9	+24	*28	.	.	.	.	.	.	.
Ranunculus repens L.	− 8	−11	+ 2	+	*15	.	.	.	.	.	.	.
Rubus fruticosus L.	−14	−11	+ 9	+ 7	+	+	*30	.	.	−14	−12	.
Rumex acetosa L.	− 8	.	+12	+ 3	*30	.	.	.	.	.	.	.
Saxifraga tridactylites Hort.	+ 9	+20	+ 1	+	*11	−14	.	.	−20	−10 +29	+16	+14
S. hypnoides Hort.	+28	+	+ 1	+	*7	.	.	.	.	−29	−16	+ 3
Sisymbrium alliaria Scop.	+17	+22	+21	*24	.	− 6	−20	− 1	.	−29	.	− 3
Tussilago farfara L.	+ 2	+	*3	.	.	.	−12	−10	+17	+14	+	+ 1

For explanation, see Table I.

Table III. *Indirect flowering from the period of maximum vegetative growth. Times of flower bud formation and of flowering*

Name of species	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Anemone nemorosa L.	+ 5	+	+	*20	.	.	−20	+5	+	+	+20	+10
Arabis hirsuta Hort.	+	+12	+	+	*27	.	.	.	+28	+14	+	+17
Auricula sp. Hort.	+ 7	*6	.	.	.	.	.	.	+24	+17	+11	+14
Eriophorum angustifolium Roth.	+	+27	+	+	*17	.	.	.	+14	+14	+	+
E. vaginatum L.	+	+	+	*29	.	.	.	.	.	+14	+	+
Fragaria vesca Hort. Royal Sovereign	+	+ 2	+	+ 3	+	*15	.	− 1	.	+23	+	+
Ilex aquifolium L.	+	+	+	+	+	*17	.	+15	+ 6	+	+	+
Primula wanda Hort.	+	+	+	*1	.	.	.	.	.	+ 1	+	+
P. veris Hort.	+	+	+	+	*10	.	−15	+19	+	+ 1	+	+
Salix caprea L.	+	+	*11	.	.	−15	+ 9	+	+12	+	+	+
Vaccinium myrtillus L.	+	+27	+	+	*6	.	+17	+	+	+	+	+16

For explanation, see Table I.

Table IV. *Cumulative flowering. Times of flower bud formation and of flowering*

Name of species	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Plantago lanceolata L.	+	+17	+31	+	*15	.	.	.	.	.	+ 8	+10
P. media L.	.	.	.	+23	+ 7	+	*2	.	.	.	.	.
P. major L.	.	.	− 7	+12	+22	*25	.	.	.	.	.	.
Taraxacum officinale Weber	+	+	+	*30	.	−28	.	.	.	.	+ 9	+

For explanation, see Table I.

Table V. *Relative sizes of leaves and flower initials in* Plantago major, *on 22 May 1938, four weeks before flowering*

Leaf no. ...	1	2	3	4	5	6	7	8	9	10	11	12	13
Length in mm.	100	165	168	198	225	232	250	223	202	127	84	49	15
Nature of bud in axil	—	Veg.	Veg.	Veg.	Veg.	Fl.	Fl.	Fl.	Fl.	Fl.	Fl.	Fl.	Fl.
Length in mm.	—	1·5	1·5	2·0	12·0	1·5	3·0	22·0	8·0	5·0	2·5	1·0	0·5
Drawing in Fig. 7 labelled		A			B		C	D					E

All the inflorescences emerged together, about 25 June. Leaf no. 1 was outermost.

The Manchester Guardian, 22 September 1938. The species had emergent or opened bloom before the end of November. Many species in the table normally flower for a period during the summer, making their flower initials singly or at intervals, and developing them into open bloom. Their late blooming is but an extension of normal behaviour, in response to the summer-like climate of the autumn. The species marked * in Table VI, however, are known to make their flower buds in late summer,

Table VI. *Plants flowering abnormally between September and November* 1938

Apple (Lane's Prince Albert)*	Honesty
Auricula*	Laburnum*
Berberis stenophylla	Lupin
Brompton stock	Magnolia
Broom (*Cytisus europeus*)*	Ox-eye daisy (*Chrysanthemum leucanthemum*)
Campanula garganica	Polyanthus (*Primula variabilis*)*
Cotoneaster microphylla	Primrose (*Primula vulgaris*)*
Cowslip (*Primula veris*)	Pyrethrum
Daphne Mezereum	Raspberry*
Delphinium	Rhododendron*
Doronicum	Rose (garden)
Erigeron (garden)	*Salix caprea**
*Forsythia suspensa**	*Trollius europeus*
Helleborus foetidus	Wallflower

Species marked * make their flower buds in late summer, for emergence the following spring (see text).

for emergence in the following spring. Abnormal flowering in this case represents the precocious development of flower initials already present; they emerged in September, October or November, instead of the following April or May. It may, therefore, be concluded that the dormant period or essential rest period, if any, of the flower bud in these species is physiologically concluded before November. The normal extension of the rest period until April or May of the following year must therefore be due, either wholly or largely, to the effects of climate.

The open weather of autumn 1938 also appeared to affect the relative stage of development of flower initials which did not actually emerge.

Thus inflorescences of *Eriophorum angustifolium* were, on an average, 2·2 cm. long on 27 December 1937, but were 9 cm. long on 12 November 1938. This observation is in agreement with results published for bulbs (e.g. Hartsema *et al.* 1930, 1932).

The effect of plant habit upon the time of flowering

It might be thought that the habit of a plant could exert an influence upon the time of flowering. Thus biennials and perennials, which often have a store of food, might perhaps be expected to flower earlier than annuals, which need to make all their food in the same short season in which they flower. Table III shows that some herbaceous perennials can make their flower initials in the year before they emerge. This question was investigated statistically, by the preparation of frequency curves showing the number of species flowering in each month of the year. The British flora, as set forth in the *Botanist's Pocket Book* of Hayward & Druce (17th ed.), was so treated, and the plant forms and times of flowering are those specified in that volume.

The results are shown graphically in Fig. 8, and reveal a maximum number of species flowering in July, for all plant habits except corms, bulbs, trees and shrubs. Bulbs and corms have stores of food, and show maxima between April and May, and in September. Their avoidance of growth in summer is perhaps a reaction of their limited root systems to relative drought at that time. They show the same relation to season in the southern hemisphere, and do not flower at the actual times which were suitable in the northern half of the globe. Periodicity must, in fact, be shifted about half a year when bulbs are taken from the northern to the southern hemisphere (Hartsema & Blaauw, 1935).

Trees reveal a maximum in May, shrubs in June, and herbaceous perennials in July. This suggests that total size of plant, with its corresponding store of food, can affect the time of flowering. A consideration of fifty-two herbaceous perennials about which data has been accumulated also confirms the view that relative food supply is linked with time of flowering. Eighteen of the fifty-two species flowered in the month of May or before; all began to form their flower initials in the previous year, and all were either evergreen, or had stores of food in rhizomes or taproot. Thirty-four species flowered in the month of June or later. Twenty-six of these were only represented in winter by a dormant crown with fine roots, that is they had little stored food. Seven species had stores of food, and one was evergreen. All thirty-four species formed their flower

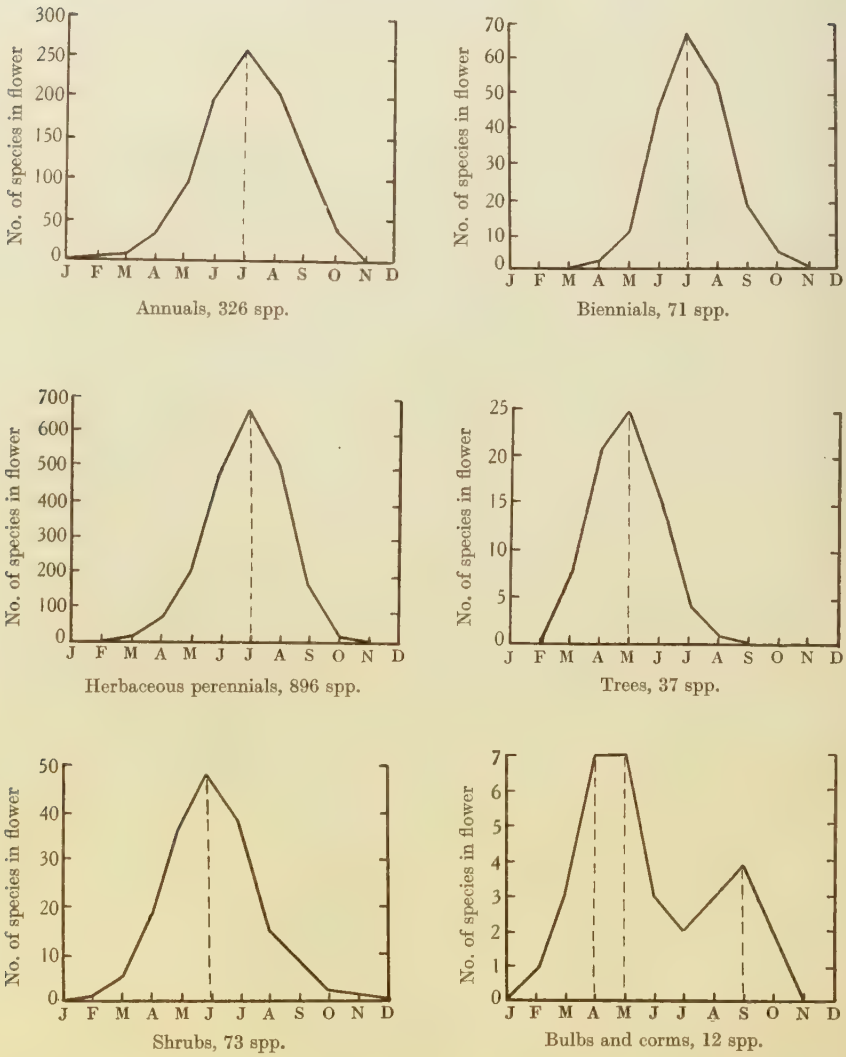


Fig. 8. Frequency diagrams for the flowering of plants of varying habit included in the British flora. Horizontal scales represent months of the year.

buds in the current year. It therefore appears that plants which are evergreen or have stores of food do not necessarily flower early, but that in order to bloom in the earlier months of the year a plant must either have stores of food, or bear functional green leaves through the winter, and must also initiate flower formation some time in the previous year or during the winter season. Fig. 8 suggests that the majority of wild herbaceous perennials and biennials of the British flora have, in general, inadequate stores of food for early flowering. They have no advantage over annuals, so far as their habit *per se* is concerned.

The question of food supply also appeared to exert an effect upon the flowering of *Dianthus Allwoodii*. Unsightly clumps of this plant, trimmed in January 1936 and 1937, failed to flower in the following seasons, while untrimmed clumps flowered profusely. It was thought that trimming had removed flower initials already made, but subsequent dissections showed that they were not present until the end of March (Table I and Fig. 3). Trimming removed the larger shoots, and left only small ones, which could evidently not form flower initials when the proper time arrived, an inability most probably due to insufficient food.

THE EFFECT OF CLIMATE PRIOR TO FLOWER EMERGENCE

Researches upon the treatment of various bulbs for early flowering have shown that temperatures at all periods between the initiation of flower formation and blooming may affect the time at which the flower emerges (Blaauw *et al.* 1932; Hartsema *et al.* 1930, 1932). Little work appears to have been done in this connexion with other plants, but the Royal Meteorological Society has maintained a large-scale survey of the phenology of thirteen wild plants since 1891, and fourteen since 1925 (Mawley, 1891–1910; Clark *et al.* 1911–34). This mass of data, with its corresponding figures for temperature, has been used to prepare a series of correlation diagrams of the two variables, temperature and time of flowering. Correlations have been prepared for each species for every month of the year, and for several meteorological districts of the British Isles. Typical diagrams, for coltsfoot in the Midlands, are portrayed by Fig. 9, whilst a larger number of results is shown in Tables VII and VIII. Correlation of these results with times of formation of the flower initials suggests the important conclusion that temperatures *prior to*, as well as after, the initiation of flower bud formation in some plants, e.g. ivy, coltsfoot and others, exert an effect upon the time of flowering. It is most noticeable that temperatures during some months (e.g. July to October

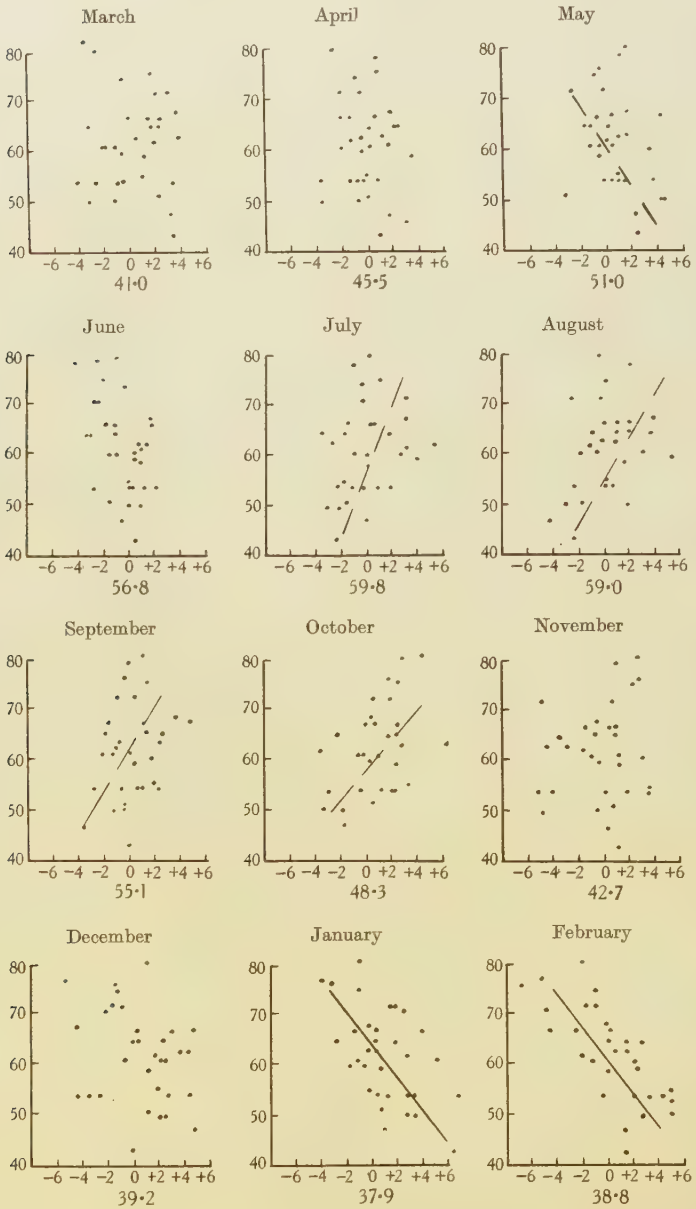


Fig. 9. (For legend see p. 699.)

Table VII. A. *Qualitative temperature correlations for the Midlands of England.*
B. *Approximate time of flower bud formation and of flowering*

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Hazel	A	\	\	...	...	...	\	...	...	...	...	...	...
	B	+	*				−	+	+	+	+	+	+
Coltsfoot	A	\	\	...	...	\	...	/	/	/	/	...	...
	B	+	*				−	−	−	+	+	+	+
Wood anemone	A	...	\	\	...	...	...	/	/	/	/	...	...
	B	+	+	*			−	+	+	+	+	+	+
Blackthorn	A	\	\	\	...	...	...	...	...	...	...	...	...
	B	+	+	*				−	−	+	+	+	+
Garlic hedge-mustard	A	...	\	\	\	...	...	...	...	...	...	...	...
	B	+	+	+	*					−	−	−	−
Horse chestnut	A	...	\	\	\	\	\	...	...	...	...	...	...
	B	+	+	+	*	−	−	+	+	+	+	+	+
Hawthorn	A	...	\	\	\	...	\	...	...	...	...	...	...
	B	+	+	+	*			−	−	+	+	+	+
White ox-eye daisy	A	...	\	\	\	\	...	...	...	...	...	...	...
	B		−	+	+	*							
Dog rose	A	...	\	\	\	\	...	...	...	...	...	...	...
	B				+	*							
Black knapweed	A	...	...	...	...	\	\	...	...	...	...	...	...
	B				−	+	*						
Harebell	A	...	...	...	...	...	\	...	...	...	...	...	...
	B					−	*						
Greater bindweed	A	...	...	...	...	\	\	...	...	...	...	...	...
	B				−	+	*						
Devil's-bit scabious	A	...	...	...	...	...	...	...	...	...	...	...	...
	B			−	−	−	+	*					
Ivy	A	...	...	...	...	\	\	...	...	\	...	...	...
	B					−	−	+	+	*			

The symbols \ and / indicate the trend of the dots in the correlation diagrams. \ means that flowering is hastened by temperatures warmer than the average, and / that bloom emergence is hastened by cooler temperatures than normal. The symbol ... shows that a correlation diagram has been prepared, but reveals no effect of temperature, i.e. the dots of the correlation diagram are either random, or their trend is parallel to one axis.
* indicates flowering, + that a flower initial or bud is present, − that no flower bud has been observed.
Averages for 1902-34. An actual series of correlation diagrams for coltsfoot is shown in Fig. 9.

Fig. 9. Correlations between the two variables, temperature and time of flowering, for the coltsfoot in the Midlands of England. The vertical scale of each diagram represents the time when flowering took place, counting the number of days from 1 Jan. 50, for instance, is 19 Feb., the 50th day of the year. The horizontal scales give temperature variations from the average "0", the actual values for which are stated in °F. for each month, below the zero. The dots portray data from 1902 to 1934, one dot for each year. Trends of the correlations are shown by oblique lines in the months of May, July to Oct., Jan. and Feb. A line sloping forward (/) indicates that flowering (in the succeeding Feb. or Mar.) is retarded by temperatures higher than normal, whilst a line sloping backward (\) means that flowering is hastened by warmer temperature. Thus higher temperatures than the average in May, in Jan. and Feb., and lower temperatures than the average, from July to Oct., hasten the appearance of the flower in the following Feb. or Mar. Leaf formation of coltsfoot in the Midlands begins in Mar., and persists until Sept.; flower buds first appear in mid-Sept., and emerge in the following Feb. or Mar.

for coltsfoot in the Midlands of England) are too high for the quickest emergence of the flower in the subsequent season. Data for the north of Scotland are somewhat scanty, but it is perhaps significant that no such temperature correlations are there observable for coltsfoot in the late summer months. The naturally lower temperatures in Scotland at this time appear to be more suited to the needs of this plant.

Table VIII. A. *Qualitative temperature correlations for the south-west of England.*
B. *Approximate times of flower bud formation and of flowering*

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	D
Hazel	A	\				...	\	...	...	...	...	...	·
	B	*					−	+	+	+	+	+	-
Coltsfoot	A	\	\			...	...	/	/	/	...	...	..
	B	+	*				−	−	−	+	+	+	-
Wood anemone	A	...	\	\		...	...	/	/	/	/	...	..
	B	+	+	*			−	+	+	+	+	+	-
Blackthorn	A	\	\	\			...	...	...	...	...	...	..
	B	+	+	*				−	−	+	+	+	+
Garlic hedge-mustard	A	...	\	\	\		...	...	/	/	/	...	..
	B	+	+	+	*			−	−	−	−	−	-
Horse chestnut	A	...	\	\	\	...	...	/	...	...	...	...	..
	B	+	+	+	*		−	+	+	+	+	+	+
Hawthorn	A	...	\	\	\		...	...	...	/	/	...	..
	B	+	+	+	*			−	−	+	+	+	+
White ox-eye daisy	A	...	\	\	\	...	...	...	...	...	...	...	..
	B		−	+	*								
Dog rose	A	...	\	\	\	\	...	...	...	...	...	...	..
	B		−	+	+	*							
Black knapweed	A	...	...	...	...	\	...	...	...	...	...	...	..
	B			−	+	*							
Harebell	A	...	...	...	...	...	\	...	...	...	...	...	..
	B						*						
Greater bindweed	A	...	...	...	...	\	\	...	...	...	...	...	..
	B				−	+	*						
Devil's-bit scabious	A	...	...	...	...	...	...	\	...	...	...	...	..
	B					−	+	*					
Ivy	A	...	...	...	...	\	...	...	...	...	...	...	..
	B					−	−	+	+	*			

For explanation, see Table VII. Averages for 1909–34.

Another feature of Fig. 9 and Tables VII and VIII is the pronounced effect of temperatures higher than the average in the months immediately before flower emergence. This effect is seen 3 months before blooming in many species, and 4 months before in the dog rose. Experimental evidence has also been obtained for the hastening of daffodil bloom emergence under conditions warmer than normal (Grainger & Crawshaw, 1939).

The results suggest that it should be possible to exercise horticultural control of temperature both prior to, and during, flower-bud initiation, and also for a time before flower emergence.

DISCUSSION

The chief results established by the present investigation are that many plants begin to form their flower initials during the period of minimal vegetative growth (Table II), and that temperatures *prior to* the floral differentiation of the growing point can affect the time of flower emergence (Tables VII and VIII). The latter finding might conceivably be explained upon the assumption that the temperatures affected the relative rate of photosynthesis, and therefore the relative amounts of food available when the flower initials began to be formed. Times of flower emergence were not always advanced by temperatures higher than normal; the reverse was often the case. This fact does not militate against the idea of a direct causal relation through the vigour of photosynthesis, since more carbohydrate may be translocated from leaves at the lower temperature than from leaves choked with carbohydrate under the warmer conditions. Initiation of the flower in the depth of winter, however, does not seem to bear any relation to external influences.

Some plants which flower indirectly from the period of maximum vegetative growth can bloom abnormally in a mild autumn. Their physiological rest period, if any, would seem to be concluded before this time, and the normal extension of dormancy until the following spring is apparently a direct effect of winter climate. This should be correlated with statements by Chittenden (1939) that development of the young flower in such plants continues slowly for a time into the autumn. The rest period may possibly be much shorter than is commonly supposed.

Cumulative flowering is interpreted as a direct response to temperature; flower buds formed in regular succession all emerge together when the temperature becomes sufficiently high.

Plant habit does not appear to affect the time of flowering, unless associated with a store of food, but flower emergence in thirteen wild plants is hastened by a temperature higher than that of normal climate at the time of opening (Tables VII and VIII). This effect can often be seen up to 3 months before blooming, e.g. blackthorn, garlic hedge mustard, horse chestnut, hawthorn, white ox-eye daisy, and dog rose. Experimental evidence of the hastening of daffodil bloom emergence by temperatures higher than normal has been obtained by Grainger & Crawshaw (1939). It would appear from the data here presented that in many plants, flower bud formation and flower emergence are governed by separate conditions of temperature.

The general conclusion from these results is that flower formation and emergence are part of an innate rhythm which can be affected to a certain limited extent by external conditions at various times. It does not seem possible to align the data here presented with any cause of flowering entirely or largely dependent upon external factors, nor with the usual conception of the carbohydrate-nitrogen ratio hypothesis. The one consistent channel of evidence is that flowering cannot take place unless sufficient food is available. Thus, it appears that a plant is not likely to flower early in the year unless it is evergreen or has stored food. It must, moreover, usually begin the formation of its flower initial in the previous year or during the winter. The removal of large shoots of *Dianthus Alwoodii* 3 months before the time when flower initials should be initiated prevented their formation. This is interpreted as the removal of food in the shoots below the level of that required for the formation of flower buds. The effect upon the time of bloom emergence of temperature prior to flower initiation is likely to be an influence upon food manufacture. Since food stored in underground vegetative organs can be adequate for the entire needs of flowering, as in coltsfoot, it seems likely that it is, at the outset, sufficient carbohydrate food that is required (cf. also Sheard, 1938), though a certain general balance of nutrients would probably be necessary for the quickest accomplishment of floral growth. Given adequate supplies of food, a plant can flower early (though not all species with stored food do so), but limited food prevents early blooming.

Summary

1. The growing points of more than 100 plant species of diverse habit have been examined between 1937 and 1939, in order to find the exact times of flower initiation.

2. Types of organization for flowering may be classified into (*a*) direct-flowering plants, (*b*) indirect-flowering plants, (*c*) cumulative-flowering plants and (*d*) climax flowering plants. Initiation of the flower in (*a*), (*b*) and (*c*) may either begin (i) during the period of maximum vegetative growth or (ii) when vegetative growth is at a minimum. In the latter class, any direct cause of flowering operated entirely or largely by external influences, such as those affecting the vigour of photosynthesis, would appear to be eliminated.

3. Abnormal flowering of some indirect-flowering plants during the autumn of 1938 suggests that their physiological rest periods were

concluded at that time. Their normal extension of dormancy until the following spring is probably a direct effect of winter climate.

4. Plant habit, as annual, biennial, perennial, does not appear to affect the time of flowering, unless associated with a store of food, but not all plants with stored food flower early.

5. A series of correlation diagrams of the two variables, temperature and time of flowering, has been prepared from the Royal Meteorological Society's phenological data. These suggest that temperatures *before* the initiation of flower bud formation, in addition to those at other periods of floral development, may affect the time of flowering. Some species appear to be hastened by higher temperatures than normal at this period, e.g. ivy, and yet other kinds by temperatures lower than normal, e.g. coltsfoot.

6. Flower emergence in thirteen wild plants is hastened by a temperature higher than that of normal climate for a period just before the time of opening.

The writer acknowledges with great pleasure the consistent help of Mrs M. Grainger and of Miss J. Grainger in all stages of this investigation; Prof. J. H. Priestley of Leeds University kindly allowed the use of a binocular microscope and gave other facilities in his Department; Mr Wilfred Crawshaw assisted with dissections of the dandelion, and Mr C. Ridgwick obtained supplies of many wild plants.

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INTRANUCLEAR INCLUSIONS IN VIRUS INFECTED PLANTS

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(With Plate XXXIX)

Introduction

Characteristic intracellular inclusions, both crystalline and amorphous, have been described in plants and animals infected with different viruses. In plants, with one exception, all the inclusions have been extranuclear. By contrast, some thirty virus diseases of animals are known in which specific inclusions are frequently found in the nuclei. The example in plants was found by Goldstein (1927) in a dahlia plant suffering from mosaic. It was an amorphous body of the X-body type, and as only one was seen can hardly be regarded as a characteristic feature of this disease. The absence of intranuclear inclusions in virus-diseased plants has afforded a striking difference between the virus diseases of animals and plants. In studying plants infected with severe etch virus, however, I have found that intranuclear inclusions are always present.

Although severe etch virus was first described by Johnson (1930) it has been little studied. In 1938 a culture of this virus was obtained by the courtesy of Dr W. M. Stanley. This virus causes in tobacco plants a severe chlorosis and greatly reduces the growth. Sometimes there is also great deformity, the leaves being reduced almost to the size of tendrils. In tobacco and all other solanaceous plants infected with this virus, two kinds of intracellular inclusion have been found. One is an amorphous, cytoplasmic inclusion similar to the X-bodies which have been described in many other virus diseases of plants. The other is an intranuclear inclusion and appears to be crystalline. The nuclei of plants infected with severe etch virus contain these plate-like inclusions with such constancy that they afford a valuable diagnostic character.

[1] Work done during the tenure of a Scholarship of the British Council.

Distribution and properties of the inclusions

Protein crystals have been described in the nuclei of a number of plant species believed to be healthy, especially in the Scrophulariaceae, but no members of the Solanaceae are mentioned in Tischler's list (1921) of plants containing intranuclear crystals. In the present work no crystals or crystalloids have been found in the nuclei of any healthy solanaceous plants or in those of plants infected with viruses other than severe etch.

Whether or not some animal viruses cause the production of intranuclear inclusions depends on the species of the infected animal and on the tissues infected. Some viruses cause their production in one type of cell but not in others, and other viruses may cause their production in one animal but not in another equally susceptible species. The age of the animal, the route of inoculation, and the virulence of the virus may all play parts in determining the formation of intranuclear inclusions. With the severe etch virus no such variation is obtained. All susceptible species of plants examined regularly produce the intranuclear inclusions. They are also present in every tissue of the infected plants, except at the growing points of roots and stems. In cells immediately below the growing points where cell division has ceased the inclusions can be found. It is possible that the great stunting of infected plants may be a result of this change in the nuclei.

The easiest method of detecting the intranuclear inclusions is examination of epidermal strips taken from below the veins on the undersurface of leaves showing pronounced chloroses. In such strips the nucleus of every cell may contain these abnormal inclusions. Sheffield (1936) found no inclusions in the guard cells of plants infected with several viruses including that of aucuba mosaic. Neither did she find any protoplasmic connexions between these and the other epidermal cells. She suggests that the virus moves from cell to cell along the plasmodesmata and suggests that the absence of inclusions in the guard cells is a result of the inability of the virus to enter them. In epidermal strips from severe etch plants the guard cells invariably contain intranuclear inclusions, but no cytoplasmic inclusions of the X-body type, which are common in other cells, have been seen (Pl. XXXIX, fig. 3). The intranuclear inclusions are found in all tissues more commonly than the X-bodies. Both types of inclusion occur in the following hosts infected with severe etch: *Nicotiana tabacum*, *N. sylvestris*, *N. glutinosa*, *Solanum*

lycopersicum, *Datura Stramonium*, *Hyoscyamus niger*. No inclusions have been seen in healthy plants of these species.

They appear to be produced equally readily in young and old plants. In the leaves, their production is restricted to those portions which show external symptoms. Leaves mature at the time the plant is inoculated rarely show symptoms: they contain little or no virus and no inclusions. On the other hand, if such mature leaves are rubbed with concentrated inoculum they become chlorotic, have a high virus content, and also contain the characteristic inclusions.

I have found that the severe etch virus is readily transmitted by *Myzus persicae* Sulz. and *M. circumflexus* Buckt. The relationships of this virus with its insect vectors seem to be similar to those described by Watson (1938) for *Hyoscyamus* virus 3 and *M. persicae*. The aphides soon cease to be infective after feeding on the source of the virus, and their efficiency as vectors, measured by the percentage of successful transmissions with single aphides, is increased by causing the insects to fast before feeding on the source of infection. Their efficiency also decreases the longer they are allowed to feed on the infected plants, more infections being obtained with insects which have fed for 2–5 min. than with those which have fed for 30 min. or longer. The intracellular inclusions, both intra- and extranuclear, are produced equally in plants infected by means of aphides and in those infected by rubbing.

The cytoplasmic inclusions are much more diffuse than the X-bodies produced by infecting *Solanum nodiflorum* with tomato aucuba mosaic virus (Henderson Smith, 1930). Most cells contain only one, but two have been seen on several occasions (Pl. XXXIX, fig. 1). They are definitely granular, appear to be aggregates, and their structure suggests that they have been built up by the aggregation of smaller particles as observed by Sheffield (1931) in aucuba mosaic. Sometimes, the X-bodies are almost spherical, when their diameters may vary between 6 and 25 μ. More often, they are shapeless, elongated masses, when their length may be as much as 50 μ.

By contrast, most nuclei contain more than one of the crystalloid type of inclusion, and as many as fifteen have been seen in one nucleus. All these are thin rectangular plates. Their size depends on the size of the nucleus and on the number of crystals present, the length of their sides varying from 3 to 10 μ. When examined between crossed Nicol prisms they show no extinctions when viewed along any axis, but whether this is because they are really optically isotropic or because they are too small to give a visible effect cannot be stated.

The intranuclear inclusions usually appear about 12–16 days after infection, that is, about a week after external symptoms become obvious. The exact time depends on the size of the inoculated plants and on the environmental conditions. The X-bodies do not appear until a week after the plate-like inclusions are obvious in the nuclei. Both types of inclusion have been seen in the roots of diseased plants, but they appear here much later than in the leaves. Once formed, the inclusions persist for long periods, and they have been found in large numbers in plants as long as $4\frac{1}{2}$ months after infection.

Before the production of the crystals, the nuclei lose their regular outline, and their contents become more granular. The crystals appear to increase in size in a manner analogous to crystal growth in saturated solutions. The nucleoli are unaffected by the production of the intranuclear inclusions, and can be differentiated, apparently unchanged, in nuclei containing many crystals (Pl. XXXIX, fig. 4).

All microchemical tests have been made on epidermal strips as these contain most inclusions and are readily penetrated by the reagents. Both kinds of inclusion give Millon's and the xanthoproteic reaction for proteins. The nuclei of infected cells stained with Feulgen's reagent, but the amorphous inclusions and the intranuclear inclusions did not. With osmic acid the intranuclear inclusions stain brown, and with iodine in potassium iodide they stain yellowish brown. The crystals are unaffected by the usual methods of decalcification of fixed tissues.

Like the X-bodies produced by strains of tobacco mosaic virus (Sheffield, 1939) the amorphous inclusions disintegrate rapidly when pressure is applied to them. The intranuclear crystals are much more stable than either the cytoplasmic inclusions or the crystalline inclusions regularly found in plants infected with tobacco mosaic virus. When epidermal strips are mounted in water and teased with needles the crystals can be extracted from the cells (Pl. XXXIX, fig. 5). Such extracted crystals are insoluble in water, alcohol, ether, and chloroform. They dissolve in 1 % acetic acid, and when pressed with needles turn into amorphous masses. The refractive index is approximately 1·51.

The most suitable fixative for the intranuclear inclusions was found to be formol-saline (20 parts formalin, 80 parts 0·9 % sodium chloride solution). Fixatives containing acetic acid, picric acid or alcohol do not give good results as they coagulate the proteins. Also, the first dissolves the crystals. Flemming's solution without acetic acid gives good results. The crystals stain readily with acid dyes such as acid fuchsin and eosin. The simplest method for differentiating the intranuclear crystals is to mount an epidermal strip in an aqueous solution of eosin. The best

Fig. 1.

Fig. 3.

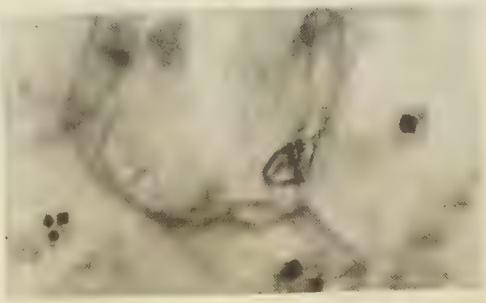

Fig. 4.

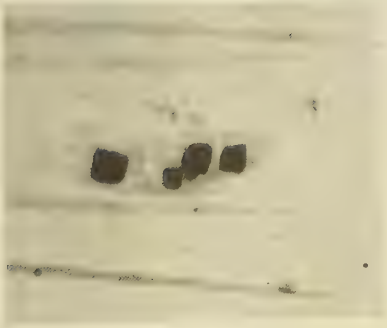

Fig. 2.

Fig. 5.

KASSANIS.—Intranuclear Inclusions in Virus Infected Plants (pp. 705–709)

method for staining fixed preparations is that of Kull, using acid fuchsin, toluidin and aurantia, which was used by Paillot (1926) in working with the polyhedral disease of silkworms. The crystals and the nucleoli stain red and the chromatin blue. With Heidenhain's haemotoxylin, the crystals stain black (Pl. XXXIX, fig. 2), and when placed in solutions of iron alum retain their colour longer than any other of the cell contents. In many ways these intranuclear inclusions accompanying infection with severe etch virus resemble the polyhedral inclusions found in infected silkworms. They are of a similar size, of the same stability, and react with stains in the same manner.

SUMMARY

Two kinds of intracellular inclusions in solanaceous plants infected with severe etch virus are described. One occurs in the cytoplasm and is similar to the X-bodies found in many other plant virus diseases. The other occurs only in the nuclei. These intranuclear inclusions appear to be crystalline, have the form of thin rectangular plates, and resemble the inclusions described in the polyhedral disease of silkworms more than any other type of previously recognized virus-inclusion.

I have much pleasure in expressing my gratitude to Mr F. C. Bawden for his help and encouragement.

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EXPLANATION OF PLATE XXXIX

Fig. 1. Epidermal unstained living cells from tobacco infected with severe etch virus showing two granular X-bodies and a nucleus containing a number of crystalline plates. ×400.

Fig. 2. Cell similar to that in fig. 1 fixed in formol-saline and stained with haematoxylin showing five intranuclear inclusions. ×900.

Fig. 3. Guard cells fixed and stained as in fig. 2, showing the intranuclear crystals. ×900.

Fig. 4. Pith cells of stem fixed and stained, infected with severe etch virus showing crystalline plates in the nuclei. Nucleoli seem to be unaffected. ×400.

Fig. 5. Single crystal extracted from the nucleus. ×900.

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THE MORPHOLOGY AND BIOLOGY OF THE BRAMBLE SHOOT-WEBBER, *NOTOCELIA UDDMANNIANA* L. (TORTRICIDAE)

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(With Plate XL and 10 Text-figures)

CONTENTS

Introduction

Notocelia uddmanniana L. is one of the most easily recognized tortricids, both in the larval and adult stages. The sluggish, dull brown larva has been found only on plants within the genus *Rubus*. On these it weaves a characteristic web, and this habit has provided the popular, descriptive name of the "Bramble Shoot-Webber" (Massee, 1937). The adult is readily identified by the large, rounded-triangular, reddish brown spot towards the lateral edge of the posterior margin of each forewing.

According to Meyrick (1927) *Notocelia uddmanniana* is a cosmopolitan species. Its range extends throughout Europe to Syria and North Persia,

also into North Africa. It has been recorded from all parts of the British Isles (Barrett, 1907; Blair, 1925; Carpenter, 1920).

Hitherto, no attempt has been made to discover in detail the habits of this tortricid. Literature on the subject is extremely scanty and consists either of short notes from collectors, stating the date and locality of adult captures (Burras, 1923), or from economic entomologists (Massee, 1937; Theobald, 1910) giving an account of the damage to cultivated Rubi. As far back as 1910 Theobald (1910) noted the damage caused by the larvae to loganberry canes. Since that date the acreage of cultivated varieties of *Rubus* has increased enormously and the moth has spread similarly. To-day, in certain fruit-growing districts of this country, it is regarded as a pest.

For the past two years the writer has undertaken a detailed study of the life history at East Malling. Observations have been made at all stages, both in the laboratory and under natural conditions.

One difficulty has been to give critical dates for any phase of the life cycle, as all stages are spread over a period of 3–4 weeks, e.g. the first adults emerge towards the end of June, but emergence is not complete until the third week in July. Nevertheless, it is hoped the results of this investigation will provide sufficient information to combat this tortricid in areas where serious damage is caused by the larva.

The adult moth

Meyrick's (1927) description of the adult is as follows:

> Forewings dilated, costa moderately arched; whitish-brownish, striated fuscous, costa posteriorly dark fuscous strigulated whitish; angulated edge of basal patch darker; central fascia fuscous, anteriorly indistinct, ending in a large rounded-triangular dark reddish fuscous whitish edged dorsal spot; an oblique fuscous fascia before apex, hardly reaching costa; extreme apex dark reddish fuscous. Hindwings grey.

Both sexes are similarly marked, though the female may have an olive green tinge in the forewings, making the general colours darker. The colour variations within the species are small and the size is fairly constant—the wing expanse of the male being 15–19 mm., that of the female 16–20 mm. (Pl. XL, fig. 1).

Earliest records for emergence during the past three years are: 21 June 1936, when one freshly emerged moth and another empty pupal case were found; 27 June 1937; and in 1938, the first evidence of adult moths was on 25 June, when one specimen was taken at night. Obser-

vations on the empty pupal cases were more satisfactory than searching for adults.

Text-fig. 8 shows how pupation is spread over one month, beginning at the end of May. Emergence of the adult covers a slightly shorter period, beginning during the fourth week in June. Thus it is possible, in the last few days of June, to find mature larvae, pupae and adults at the same time and in the same place.

During the daytime the moth remains inactive, usually resting on the underside of a leaf, but in woodlands near wild blackberries it may be found on plants other than the host. When disturbed it darts off with a quick but erratic flight, seldom travelling more than a few feet, and again alighting on a leaf or on the ground.

Light has a certain attraction for this species, and several specimens of the adult have been taken at night in lighted rooms. A more exact measure of the flight period is given from unpublished records of captures in the Rothamsted light trap (Williams, 1935) during the years 1933–5. Attached to this trap are a series of eight killing bottles which are each in use for one-eighth of the night—from half an hour after sunset to half an hour before sunrise—then automatically changed. At midsummer each bottle is exposed for approximately 45 min., increasing to 58 min. on 1 August. The total catch of *Notocelia uddmanniana* for each period during the three years was 1, 2, 15, 6, 2, 2, 0, 1. This shows that a few moths are active at almost any period of the night, but the maximum flight occurs in the two periods immediately preceding midnight. A study of the individual dates gives the earliest capture as 23 June in 1933, and the latest 23 July in 1935. Both these dates fall within the limits observed in more recent years at East Malling.

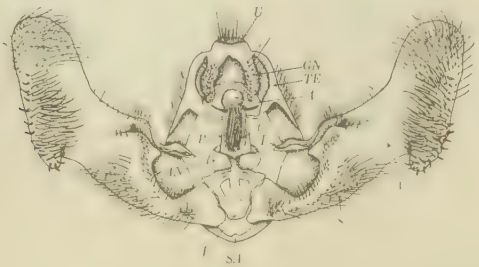
Under caged conditions the adult life varies from 10 to 19 days with an average of 14–15 days (Table III). Other moths which were sleeved on to the tips of young canes in the field—this being the nearest approach to natural conditions possible—lived from 9 to 15 days with 12 days as an average. No appreciable difference has been noted in the length of life of the two sexes, though it was not uncommon for caged females to outlive the males by 1 or 2 days.

Genitalia of the male

In the following description, the nomenclature used by Pierce (1914) has been followed.

A distinguishing character of the genus *Notocelia* is the presence of two spines at the orifice of the aedeagus (Text-fig. 1). The uncus (*U*) is small, with many hairs at

the blunt apex. Each gnathos (*GN*) is covered, on the ventral surface, with long, slender hairs. The costal margin of each valva (*V*) is produced inwards, terminating freely in a point (*T*). The inner portion of each valva, or sacculus (*S*), is well covered with long hairs and short bristles; near the costal margin is a single, very large, blunt spine. Along the dorso-lateral edges of the valvae are numerous long hairs, changing to stouter bristles in the ventral half of the lateral margins. Many cornuti (*C*) are attached to the vesica; these appear to be easily shed, for in several preparations of the genitalia only the basal scars remained.



Text-fig. 1. Male genitalia. Ventral view (×32). *A*, anus; *AN*, anellus; *C*, cornuti; *GN*, gnathos; *J*, juxta; *P*, penis; *S*, sacculus; *SA*, saccus; *T*, transtilla; *TE*, tegumen; *U*, uncus; *V*, valva.

The egg

Shortly after deposition the egg is translucent white, very soft, somewhat irregular in shape and enclosed in a thin but much pitted shell which remains soft until hatching. Colour changes are small, the white giving way to a pale cream which is retained until the larva emerges. The developing embryo only becomes obvious about 3 days before hatching, when the darkening head capsule may be seen through the fragile shell. Viewed from above (Pl. XL, fig. 2) the egg appears circular or oval; the dorsal surface is always convex, while the ventral surface, which is attached to the leaf by a glutinous substance, takes on the configuration of the leaf surface immediately beneath it.

Eggs are laid singly, either on the dorsal or ventral surface of a leaf near the growing point of a current year's cane. The very small, almost colourless egg is difficult to find in the field, and this was only overcome by releasing numbers of adult moths on an isolated plot of *Rubus*. It was then certain that after a few days eggs would be present on the plants, and by careful searching many were located.

When caged, the female shows no preference for either leaf surface. In the field, however, only 40 % of the eggs discovered were on the ventral

leaf surface. Those deposited on the dorsal leaf surface are very flat, and invariably found in the depression above one of the veins. On the underside of the leaf the egg is more spherical but much less regular in contour. These differences in shape arise because the soft-shelled and very liquid eggs are laid on two entirely different surfaces—above, the leaf is smooth and the egg can spread until it is almost flat; beneath, the egg is laid on a mat-like surface of leaf hairs and held by adjacent hairs. In fact the pressure of occasional leaf hairs make slight indentations in the lateral surface, hence the irregularity in outline. Table I illustrates this difference, giving the measurements of thirty eggs from each side of the leaf.

Table I. *Measurements of eggs of* Notocelia uddmanniana *from each leaf surface, in mm.*

Longest horizontal axis			Horizontal axis at right angles		
Max.	Min.	Av.	Max.	Min.	Av.
Dorsal surface					
0·68	0·56	0·64	0·56	0·44	0·48
Ventral surface					
0·60	0·44	0·53	0·52	0·32	0·41

Under field conditions never more than three eggs are found on a leaf; usually there is only one, and unless the infestation is severe very few eggs are laid on each new cane. This is confirmed by observations on the larval habit; it is unusual to find more than two or three first stage larvae feeding in the same terminal bud. Pl. XL, fig. 2, indicates the size, shape and position of the egg on the dorsal leaf surface. The numerous eggs on one leaf shown in the photograph were laid by caged females, and this is very different from the usual single egg per leaf deposited under natural conditions.

Oviposition takes place entirely by night, and eggs are never deposited until the second night after emergence of the adult from the pupa. The largest number of eggs is laid the first night; then for a period of 4–5 days the numbers are about equal, after which a gradual, though not constant diminution in the number occurs until death. Having laid all her eggs the female seldom survives for 24 hr. Table II shows the egg laying of three females which emerged on 21 June 1937.

Over 300 eggs may be deposited by a single female during her life of 10–19 days in the laboratory. In the field no moth lived longer than 15 days.

Tables III and IV illustrate the egg-laying capacities of this species. Field records were obtained by sleeving equal numbers of each sex on to

the growing tip of a young loganberry cane with a muslin bag, though an objection to this method was that some adults became entangled in the muslin folds where the bag was tied around the cane and died prematurely.

Table II. *No. of eggs deposited daily by* 3 *female* N. uddmanniana

Date	No. of eggs	Date	No. of eggs	Date	No. of eggs	Date	No. of eggs
28 June	—	2 July	109	6 July	50	10 July	8†
29	138	3	117	7	48	11	0 (all dead)
30	118	4	78	8	39*		
1 July	105	5	58	9	3		

* One female died. † Second female died.

Table III. *Eggs laid by* N. uddmanniana *when caged in the laboratory*, 1937

Date emerged from pupae	No. of females	Total no. of eggs deposited	Average no. of eggs deposited	Length of life of each female in days	Average length of life, days
17 June	3	869	290	13, 17, 19	16·3
21	3	871	290	12, 15, 15	14
24	5	1387	277	10, 12, 14, 14, 17	13·4
24	3	706	235	10, 12, 12	11·3
28	3	939	313	14, 17, 18	16·3
28	6	1549	258	13, 14, 14 15, 17, 17	15
28	3	622	207	14, 15, 15, 18	14·7
1 July	4	1052	263	12, 14, 15, 18	14·75
Totals	30	7995	266·5	452	14·5

Table IV. *Eggs laid by* N. uddmanniana *in muslin sleeves in the field*, 1937

Date emerged from pupae	No. of females	Total no. of eggs deposited	Average no. of eggs deposited	Length of life of each female in days	Average length of life, days
21 June	2	414	207	11, 14	12·5
23	3	787	262	12, 14, 15	13·7
25	3	741	247	10, 14, 14	12·7
25	3	644	215	9, 12, 12	11
28	3	694	231	10, 13, 15	12·7
28	4	648	162	6,* 8,* 11 14	9·75
1 July	3	738	246	12, 13, 15	13·3
1	2	421	210·5	11, 12	11·5
1	2	394	197	10, 13	11·5
Totals	25	5481	219·2	300	12

* These females died prematurely.

It will be seen from Tables III and IV that laboratory conditions apparently favour both egg laying and length of adult life.

Period of egg laying

Adults begin to emerge during the latter part of June, and within a few days, where the larvae have been noticed in numbers previously, sufficient empty pupal cases of both sexes may usually be found to indicate that mating is possible. Egg laying continues until the end of July.

Hatching

From a batch of eggs laid during any one night the greatest hatch occurs on the second day. A few larvae hatch the first day, 65–85% hatch on the second day, and the remaining eggs all hatch by the following day, or the period may extend over 3 or 4 days.

The incubation period varies from 13 to 18 days, with an average of 15 days, in the field; in the laboratory the period is 8–11 days with slightly over 9 as the mean.

Emergence is through a longitudinal slit, cut by the larva, just above the lateral egg margin. The larva does not eat the shell, which remains attached to the leaf for many days after hatching.

The larva

Feeding habits

On hatching, the young larva immediately migrates to the growing point of the cane on which the egg was laid. Here it crawls between the dorsal halves of a slightly opened leaf, and by means of silken strands webs the two halves together. Both first and second stage larvae are commonly found in this position. As the leaf develops, and becomes tougher, the larva moves up the cane to a more tender leaf, treating it in a similar manner. Before the hibernation stage is reached most of the larvae have ceased to inhabit a single leaf and burrowed into the actual growing point of a cane. At this period they are so small that no harm to the plant results from their feeding.

When hibernation takes place in August, the larva is on the current year's cane; on emerging in the following April it is on the fruiting cane of that year, since most of the cultivated Rubi produce canes which fruit in the second year and then die. Some larvae feed on the fruiting laterals of this cane, at first webbing together leaves, then when the blossom buds

appear they tunnel into the centres of the buds, eat the interior and leave only a shell composed of sepals and petals.

In April others leave the old canes on which they spent the winter, and wander on to the young canes, now a few inches above soil level. Here the halves of a young leaf are webbed together, or two overlapping leaves may form the initial larval habitat. As the cane grows and more leaves open, the web is enlarged to encircle these leaves, until finally the whole shoot-tip is tightly drawn together with the bud completely hidden (Pl. XL, fig. 3). Larvae still use the older leaves as a shelter, making excursions to the tender leaves and bud for food. Frass is often mixed with the silk strands forming the web, and sometimes an opening remains in one of the leaves through which it is ejected.

Those larvae on the fruiting laterals do not often remain after the blossom buds have reached an advanced stage and no further new leaves are being produced. Instead, they move on to the young canes, webbing together the leaves around the growing point as do the larvae which migrate immediately on emergence from hibernation. Except when the infestation is severe, not more than one larva is found in a web. The limiting factor to this solitary existence is reached when the larvae outnumber the new canes. Then it is quite common to find as many as three or four living together in one web. Normally the larva lives in the same web on a new cane throughout its post-hibernation life, and finally pupates in the web.

Length of instars under laboratory conditions

In this experiment the larvae were reared in glass-topped tins, on a single, young loganberry leaf for the first and second instars; later the whole shoot tip was provided. Food was changed twice weekly, more frequently if necessary, and kept as fresh as possible by inserting the petiole or cut portion of the cane in a small heap of damp sand placed at the edge of the tin. Humidity was necessarily high, but no deleterious effect was noted; the temperature was fairly constant throughout the active phases of larval life, varying from 65 to 70° F., with occasional wider fluctuations. Sixty larvae were used each year, and the great advantage of this method proved to be the ease in handling the food when daily inspections were made for moulted head capsules. Also, should the capsule fall off the leaf, a clean, bottom surface of the tin made its discovery easy. Only one larva was kept in each tin.

From hatching to hibernating (Table V), under these conditions, is a

very short period; one larva commenced spinning its cocoon a fortnight after emergence from the egg.

Table V. *Duration of larval instars under laboratory conditions, in days*

	1936–7			1937–8		
Instar	Max.	Min.	Av.	Max.	Min.	Av.
I	10	7	8·5	10	5	7
II	8	4	5	8	4	5
III prior to hibernation	20	4	7	16	4	7·5
III after hibernation	10	6	7	10	6	7
IV	12	8	9·5	12	7	9
V	15	9	11	14	8	10

The larva normally hibernates in the third instar, but an occasional one was observed to spin its web in preparation for the quiescent, overwintering phase before passing through the second moult; in such cases the average duration of the second instar was prolonged, and ranged from 6 to 20 days, with an average of 12½ days. This was infrequent, for only 10 out of 120 hibernated before the third instar.

By removing the silken web forming the cocoon, it was found that these larvae had moulted again, within a short period of spinning up. Other specimens which did not moult twice before hibernation were left alone until they emerged in the following spring. In every instance a head capsule was found incorporated in the web, not loose, as would be expected had the larva moulted after beginning the resting period. This evidence suggests that moulting always occurred before the winter cocoon was completed, and within a few days of cessation of feeding.

The batch of sixty larvae placed in observation cages in the summer of 1937 produced two which went into the fourth instar before hibernating. One other pupated after moulting only four times, and a male moth emerged 5 weeks after the egg hatched. This was an exception, but such an occurrence was not confined entirely to the laboratory experiment, as will be seen when the instars are discussed under field conditions.

In the laboratory the second instar is the shortest. On an average the length of active life of the third instar is about the same before and after hibernation. Some larvae, however, feed for as long as 20 days prior to hibernating, never longer than half that period after emerging in the spring before moulting again. The third is the longest instar, but it is divided into two feeding phases, then in the fourth stage the larva lives

longer than either the first or second instars, and the final stage is a day or two longer than the fourth.

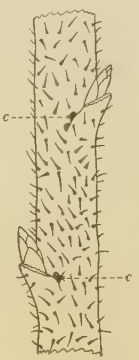
Field observations

Only approximate data can be given on the length of instar as it varies considerably between different larvae. As this species prefers a solitary existence in the spring, with only one larva in each cane tip to avoid any confusion, the number under observation on potted plants was limited to the number of young canes available.

Table VI. *Duration of larval instars in the field, in days*

	1936–7			1937–8		
Instar	Max.	Min.	Av.	Max.	Min.	Av.
I	20	13	16	20	12	15
II	13	8	10·5	13	8	10·5
III prior to hibernation	23	7	10	20	7	12
III after hibernation	21	14	16	19	12	14
IV	28	20	24	24	16	20
V	35	22	27	35	21	26

Egg laying begins at the end of June, and a fortnight later the first larvae have hatched. Thus by the last week in July many of the larvae are already in the second instar. Emergence of the adult from the pupa is spread over 3–4 weeks, hence some larvae will still be in the first stage when the earliest ones, during the second week of August, are already preparing to hibernate. By the middle of August, a great many have settled down in their winter cocoons, and none of this generation has been found as late as September at East Malling.



Text-fig. 2. Drawing to show position chosen by larva of *N. uddmanniana* to hibernate. *c*, hibernation cocoon.

Upon cessation of feeding, the larvae leave the tips of the canes and migrate towards the basal half. The position chosen for the winter resting place is always near a node (Text-fig. 2) commonly between the base of a petiole and the main cane. A slight groove is excavated in the cane, and a tough silken web spun around the outside; the entire interior is covered with a finer silky lining. Any frass voided during the preparation of this cocoon is found adhering to the outer web. Sometimes the larva may find a sheltered position under a

piece of dead epidermis; this again is frequently near the base of a petiole. About 8 months are spent in hibernation.

Table VII and Text-fig. 3 show the period over which the larvae leave their hibernating cocoons and recommence feeding. In 1937 only a small number of larvae, on potted plants, were available for observation. The data for 1938 were obtained from a plot of mixed commercial varieties of *Rubus*, where numerous adults had been released the previous July.

Each lateral and new cane was inspected every other day and any larvae found were removed. The figures in Table VII represent the total number of larvae emerging from hibernation in the 48 hr. period immediately following the previous count.

Table VII. *Emergence of* N. uddmanniana *larvae from hibernation; counts made at 2-day intervals*

Date	1937	1938	Date	1937	1938	Date	1937	1938
26 Mar.	—	—	9 Apr.	—	25	23 Apr.	—	1
27	—	—	10	18	—	24	2	—
28	—	3	11	—	18	25	—	1
29	—	—	12	13	—	26	1	—
30	—	8	13	—	12	27	—	—
31	—	—	14	7	—	28	2	—
1 Apr.	—	13	15	—	7	29	—	—
2	—	—	16	6	—	30	1	—
3	—	25	17	—	3	1 May	—	—
4	1	—	18	5	—	2	1	—
5	—	30	19	—	2	3	—	—
6	8	—	20	2	—	4	—	—
7	—	29	21	—	1	5	—	—
8	20	—	22	2	—			

In 1937, 4 April was the first date when a larva was seen. Within 10 days, 75 % had left their hibernating quarters and the remaining 25 % came out over a longer period, the last being found on 2 May. By 18 April, 14 days later than the first larva appeared, 88 % had emerged, and 2 days later 90 % were feeding.

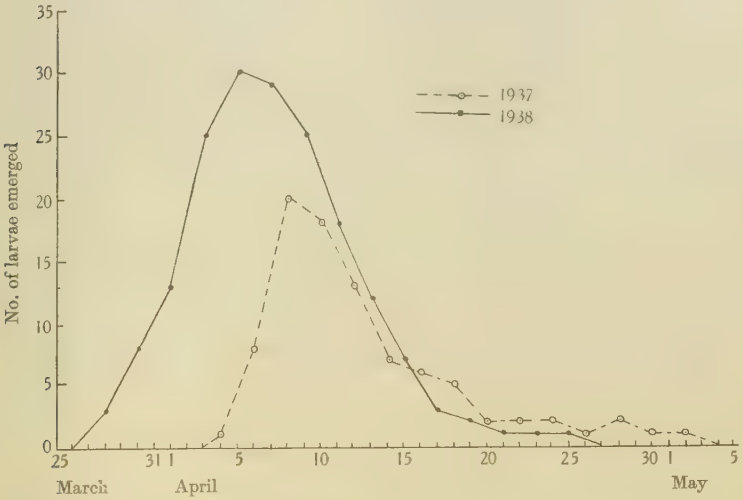
Warmer weather prevailed during the early spring of 1938, and it is possible that these favourable conditions were responsible for an earlier emergence from hibernation of the 1937–8 generation. The first record, 28 March, is a week earlier than that of the previous year. Again, Text-fig. 3 shows a rapid rise in the number emerging for the first 10 days, then an almost as rapid decline until only one or two fresh larvae appear at each inspection. This latter condition persisted for a fortnight in 1937, but only just over a week in 1938. A study of the percentage gives very similar results to the first year's readings. In 1938, 75 % of the larvae

are again feeding by 9 April, 12 days after the first emergence, and 90% 4 days later.

Thus at East Malling over 90% of the hibernating larvae of *N. uddmanniana* are feeding early in the third week of April, after emerging over a period of 16–18 days.

Emergence from the cocoon is affected by the larva biting a hole just sufficiently large to allow its body passage to the outside.

In the field, the spring stages are considerably longer than in the laboratory, no doubt due, in part at least, to the less favourable conditions prevailing outside, in particular the lower temperatures.



Text-fig. 3. Graphs to show emergence of larvae of *N. uddmanniana* from hibernation in 1937 and 1938, under field conditions.

Fourth stage larvae are present after the middle of April, and the fifth and last instar early in May. Occasional fourth instar larvae are found as late as the end of May and early in June, the result, most probably, of a parasite present in the larva and retarding development.

In 1937, larvae of this species were discovered in very small numbers, in the final instar, during the second week in August. These were brought into the laboratory and fed; they pupated almost immediately, and on 24 August two female moths emerged, together with one male, while another male appeared on 31 August. Mating took place and many eggs were laid. The resulting larvae developed and, by the end of

September, all had hibernated; this time, with few exceptions, in the second instar. Only a few emerged in 1938, but those that did, matured, taking longer to complete the spring portion of the life cycle than the single generation from the adults of the previous June.

Whether the number of pupae of this early maturing group was sufficient to produce a partial second generation under natural conditions is doubtful. A large area of this district had to be searched in order to obtain the ten mature larvae from which only five adults emerged. A search was made to discover a partial, second brood in the field, but no evidence was obtained to suggest the presence of a second generation.

A similar phenomenon was noted with another species of Tortricid, *Cacoecia podana* Scop., but again only the mature larvae and an occasional pupa could be found. There was no trace of a second generation. The summer of 1937 provided exceptionally favourable weather conditions, which may account for this abnormally early maturity of certain larvae of these two species.

Morphology

First instar.

Shortly after hatching the larva is white, with a dark brown head, and approximately 1 mm. in length. The dorsal and dorso-lateral area of the prothorax, with the exception of the anterior edge, is covered by a light brown chitinized plate—the thoracic plate. Another smaller plate, similar in colour, is present on the posterior dorsal area of the anal segment; this is the anal plate. The thoracic legs are pale grey and very lightly chitinized.

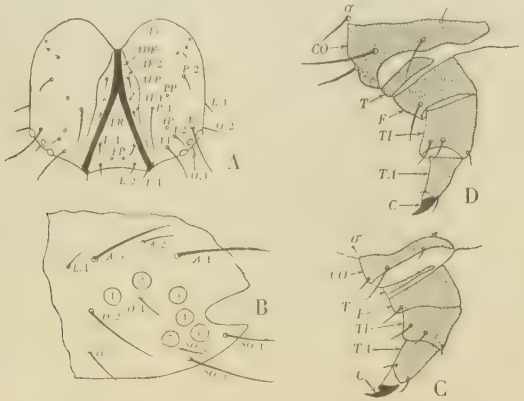
By the third day a yellowish tinge has appeared in the body colour, and this increases in intensity, until, in the fully developed first stage, the larva is orange-brown. The larva measures 2 mm. in length at the end of the instar.

Head. The head capsule (Text-fig. 4 A, B) varies from 0·19 to 0·23 mm. in width, with an average of 0·215 mm. It consists of a V-shaped median dorsal frons (*FR*), confluent with the clypeus, separated on either side by an elongate, narrow adfrontal sclerite (*ADF*) from the epicranial plates (*EP*). Two groups, each of six ocelli, are situated laterally on the anterior half of the epicranium, one group on each side. The first four ocelli form a quadrant of a circle, the fifth posterior and ventral to number four, and the sixth is the most anterior, on a line midway between four and five.

The arrangement of the head setae is normal. The anterior setae (*A* 1, *A* 2, *A* 3) are so arranged that *A* 1 is vertically above the third ocellus, *A* 2, a much smaller seta, above the second ocellus and in a higher horizontal plane than *A* 1, and *A* 3 on the same level as *A* 1, above the posterior edge of ocellus one. The anterior puncture (*AP*) is situated some distance behind *A* 1. Behind or below the ocelli are three ocellar setae (*O* 1, *O* 2, *O* 3); *O* 1 is just beneath the second ocellus, *O* 2 posterior and ventral to the first ocellus, and *O* 3 almost beneath *O* 2, near the ventral edge of the head capsule. Three sub-ocellar setae (*SO* 1, *SO* 2, *SO* 3) are also present; *SO* 1 in the anterior, ventral angle of the epicranium, a smaller seta, *SO* 2, close to the anterior,

ventral edge of the fifth ocellus, and *SO* 3 still more ventral, vertically between ocelli five and six. The posterior setae (*P* 1, *P* 2) and the posterior puncture (*PP*) lie in a straight line, diagonally across the epicranium; *P* 1 is directly in line with *AP* and *A* 2 near the adfrontal suture, *P* 2 more lateral and posterior, with the puncture midway between them. There are two minute ultra-posterior setae (*S*) and two punctures behind *P* 2. A single, small lateral seta (*L* 1) is present a short distance behind *A* 3.

On the adfrontal sclerite are two adfrontal setae, *AF* 1 in a median position near *P* 1, and *AF* 2 more posterior. An adfrontal puncture (*AFP*) lies midway between the adfrontal setae.



Text-fig. 4. A, head of first instar larva (×125). B, enlarged antero-lateral region of A. C, right mesothoracic leg of first instar larva, posterior view (×250). D, right mesothoracic leg of mature larva (×32). *ADF*, adfrons; *EP*, epicranium; *FR*, frons; *A* 1, *A* 2, *A* 3, anterior setae; *AP*, anterior puncture; *AF* 1, *AF* 2, adfrontal setae; *AFP*, adfrontal puncture; *E* 1, *E* 2, epistomal setae; *F* 1, frontal seta; *FP*, frontal puncture; *L* 1, lateral seta; *O* 1, *O* 2, *O* 3, ocellar setae; *P* 1, *P* 2, posterior setae; *PP*, posterior puncture; *S*, ultra-posterior setae; *SO* 1, *SO* 2, *SO* 3, subocellar setae. The ocelli are numbered. *C*, claw; *CO*, coxa; *F*, femur; *T*, trochanter; *TI*, tibia; *TA*, tarsus.

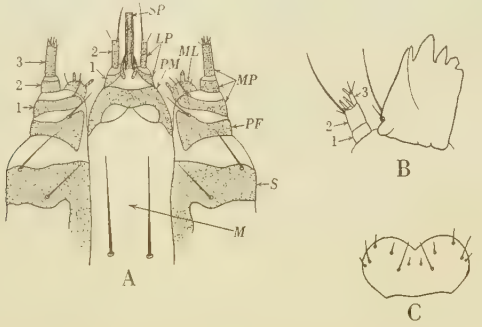
Towards the anterior edge of the frons, near each adfrontal suture are the frontal setae (*F* 1). Still more anterior and nearer the median line of the frons are a pair of frontal punctures (*FP*). Two pairs of epistomal setae (*E* 1, *E* 2) are present in the clypeal area, *E* 1 in the extreme anterior angle adjacent to the adfrontal suture, *E* 2 a little farther removed from the anterior margin, and in approximately the same vertical planes as the frontal setae.

Labrum (Text-fig. 5 C). This consists of two rounded lobes on a flat base, meeting anteriorly to form a shallow, median incision. There are three lateral setae on each side, a pair, one large and one small, near the lateral margin, the third very close to the antero-lateral edge. Three pairs of median setae are also present, a pair of small setae close to the median line, one-third of the length of the labrum from the median incision towards the base, a pair of much longer setae situated postero-laterally to the

median pair, and the third pair, short, antero-lateral, with their bases as far forward as the apex of the incision.

Mandibles (Text-fig. 5 B). The mandibles are alike, almost parallel-sided and heavily chitinized. From a postero-lateral position on the dorsal surface arises a long bristle; a small seta is found in a similar position on the ventral side of each mandible. Four teeth are present, the three outer ones pointed and largest, the fourth is blunt and considerably smaller. The inside, anterior edge of each mandible may sometimes be produced to form a small tooth, though more often it is flat.

Maxillae (Text-fig. 5 A). They consist of cardo and stipes (*S*), palpifer (*PF*), three jointed maxillary palp (*MP*), and a maxillary lobe (*ML*). Ventrally, two long, stout bristles are present on the stipes, one arising from a median position where the stipes broadens, the other near the antero-lateral edge. On the ventral, anterior margins towards the internal edge of both palpifer and the basal segment of the palp arises a stout bristle.



Text-fig. 5. Mouthparts of first instar larva (×250). A, maxillae and labium, ventral view. B, right mandible and antenna, ventral view. C, labrum, dorsal view. *LP*, labial palp; *M*, mentum; *ML*, maxillary lobe; *MP*, maxillary palp; *PF*, palpifer; *PM*, prementum; *S*, stipes; *SP*, spinneret.

On the outer side, the palpifer and two basal joints of the maxillary palp are equal in length, but ventrally the palpifer broadens rapidly towards the labium, whilst the basal segment of the palp narrows slightly. The terminal joint is three times as long as each of the other palpal segments, and bears at its apex two short spines, a pair of papillae each surmounted by a bristle, and another bristle on the interior edge.

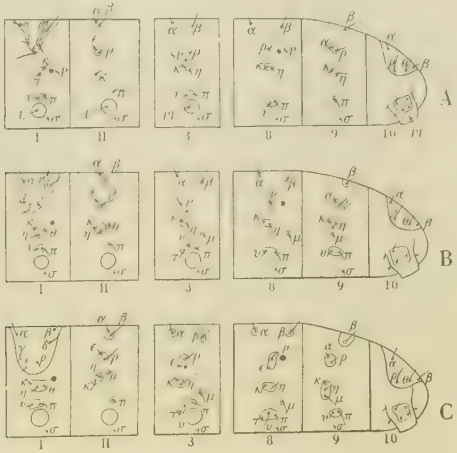
Situated on the anterior margin of the basal segment of the palp, and adjacent to the labium, is the maxillary lobe. Near its apex are a pair of two jointed papillae, between them a stout spine, and laterally, on the side nearest the maxillary palp, another spine.

Labium (Text-fig. 5 A). Median, and on the ventral side of the head, is the labium, joined to the maxillae by a membrane. The mentum (*M*) is almost membranous, and bears two very long, thick bristles, which arise on the ventral side near the basal margin. A more strongly chitinized prementum (*PM*) carries a central spinneret (*SP*) and, laterally, labial palps (*LP*). Each labial palp consists of a short, broad, basal joint, and a long, thin, terminal segment, bearing at its apex a small

papilla from which arises a very long bristle, and a shorter bristle situated laterally and distal from the spinneret.

Antennae (Text-fig. 5 B). Each antenna consists of a narrow basal joint, a longer second segment, and a short, thin, terminal joint arising from the edge of segment 2 nearest the mandible. The basal and terminal segments are equal in length.

From the external, antero-lateral edge of the middle joint arises a long bristle, and near the base of this bristle is a much shorter one. Two elongate papillae, one dorsal and the other ventral, are situated at the apex of segment 2, together with an extremely minute bristle. The distal end of the apical segment bears a short bristle, a small papilla surmounted by a bristle, and a longer papilla.



Text-fig. 6. Chaetotaxy of the larval instars. A, first instar. B, second instar. C, third instar. *L*, leg; *PL*, proleg; *SP*, spiracle. Roman numerals denote thoracic segments.

Chaetotaxy (Text-fig. 6 A). The method of designating the setae in these descriptions follows Fracker (1915). Six setae are present on the thoracic plate each side of the median dorsal line. Antero-laterally, almost on the edge of the chitinized area, is alpha, farther along the anterior lateral edge is gamma, with epsilon at the ventral extremity of the plate. Near the posterior edge of the thoracic plate are beta, slightly nearer the median line than alpha, delta, a long seta, situated at a lower level than alpha, and rho still more ventral, on a level about midway between gamma and epsilon. Beneath the ventral extremity of the thoracic plate and just anterior to the spiracle on the prothorax, two more setae, kappa and eta, arise from a common basal chitinized area. Still more ventral, a little above the leg, are nu and pi with a common base, and sigma is present on the ventral surface of the segment near the leg.

Both mesothorax and metathorax have identical setal maps. Alpha and beta arise from the same base on the dorsal surface, with alpha smaller and more dorsal than beta. Epsilon and rho have a common basal plate situated in a dorso-lateral position beneath alpha and beta. Kappa is a single seta beneath the rho group; pi

is ventral and posterior to kappa, above the insertion of the leg, and sigma is ventral, adjacent to the posterior edge of the leg.

The first eight abdominal segments are homologous in setal arrangement. Dorsally alpha and beta arise from separate tubercules, with alpha smaller, anterior and nearer the median line. Rho is large, situated near the dorsal edge of each spiracle, except in segment 8, where the spiracle is more dorsally situated, and here rho is directly anterior to the spiracle. A short distance immediately beneath each spiracle kappa and eta have a common base, with the exception, again, of segment 8, where the spiracle is farther removed from the setae and postero-dorsal to them. Very near the dorsal edges of the proleg bases on segments 3–6, and in homologous positions on the other segments, nu and pi arise from the same base, pi is the longer bristle and postero-dorsal to nu. Sigma is near the mid-ventral line, postero-ventral to the prolegs and in a similar position on the other segments.

On the ninth segment both beta setae arise from the same chitinized plate, occupying an almost median dorsal position. Alpha and rho are more anterior and lateral on a common base. Kappa and eta are together, beneath alpha and rho, eta slightly posterior and ventral to kappa. Still more ventral is pi, and sigma as on the other segments. In no first stage larva could nu be found on the ninth segment.

Four pairs of setae arise from the anal plate. Alpha and theta are on the same horizontal level with beta posterior to theta and nearer the mid-dorsal line. Rho is placed on the lateral edge in front of theta but not so anterior as alpha.

Each anal proleg has a small chitinized plate on the outside, containing three small setae and a puncture, arranged in the form of a square with the puncture in the posterior dorsal corner. There is a pair of setae in a median position on the posterior surface of each caudal proleg, another pair similarly placed on the anterior side, together with a pair on the same side just beneath the base of each proleg.

Spiracles. Nine pairs of spiracles are present in the first stage larva, one pair on the prothorax and a pair on each of the first eight abdominal segments.

Prolegs. Each proleg on the third to sixth abdominal segments possesses a uniordinal series of crochets varying from six to nine in number. Anal prolegs have five to seven crochets arranged uniordinally only along the anterior one-third of each margin.

Second instar.

The larva at this stage may be distinguished from the first instar by body colour alone—the orange tinge disappears rapidly after the first moult, and is replaced by brown. The head capsule may be shining black or dark brown; both thoracic and anal plates are darker and more heavily chitinized, though the anal plate is still paler than the thoracic one. Small chitinous areas surrounding the bases of the setae are darker brown. Orange red mandibles are conspicuous, and legs a darker grey. The head capsule varies from 0·29 to 0·39 mm. in width, with an average of 0·34 mm. When fully fed the larva measures 3–3·5 mm. in length.

Chaetotaxy (Text-fig. 6 B). Eta appears as an addition to the kappa tubercule on the mesothorax and metathorax. Each of the three thoracic segments has another seta, theta, added to the kappa group, arising from the same chitinous plate as kappa and eta on the prothorax, but on the other two segments it has a separate base, situated adjacent to the posterior margin of the kappa and eta tubercule.

Segments 1–6 of the abdomen contain tau as an addition, on the same basal plate as nu and pi, and anterior to these two setae. Nu is now present with pi on segment 9.

Dorsal and posterior to the tau group the first eight abdominal segments bear a new single seta, mu. This seta also appears for the first time on segment 9, where it arises from the same chitinous area as kappa and eta.

Prolegs. The crochets are still uniserial and uniordinal, but they are considerably larger than for the previous instar. Seven to thirteen crochets are present on each proleg except the caudal pair, where five to eight are arranged around the anterior edge only.

Third instar.

After the second moult the larva assumes the dark brown coloration which remains constant until pupation. In this stage the head capsule width of individual specimens varies from 0·46 to 0·64 mm., with an average of 0·54 mm. The head capsule is shining black, the thoracic plate more extensive than previously, either shining black or dark brown, with a paler anterior margin, and the anal plate slightly paler than the body. Around the bases of the setae the chitinous areas are more definite, as are the tubercules from which the actual setae arise. Thoracic legs which had become darker grey and more chitinized in the second instar are now almost black. The length of the larva at the end of the instar is about 6 mm.

Chaetotaxy (Text-fig. 6 C). The chitinous base from which rho arises on the first eight abdominal segments is produced anteriorly around the dorsal edge of each spiracle, and an extremely minute seta appears near the antero-ventral edge—this is epsiloh. There was a trace of this new seta on the first abdominal segment in occasional specimens of the second instar larvae, but so few possessed it that it was doubtful whether epsilon should be generally present until this stage when all eight segments carry the seta. Tau is now present on segments 7 and 8. On the mesothorax and metathorax theta has moved farther away from the base of kappa and eta, and has a much enlarged basal plate.

Prolegs. Crochets are intermediate between uniordinal and biordinal, arranged as in previous instars with ten to seventeen crochets on each of the first four pairs, and seven to twelve along the anterior edges of the anal prolegs.

Fourth instar.

There is no appreciable difference in colour from that of the third instar larva. The head capsule increases in width to 0·77 mm., ranging from 0·68 to 0·86 mm., and the larva is about 1 cm. in length when fully grown at the end of the instar.

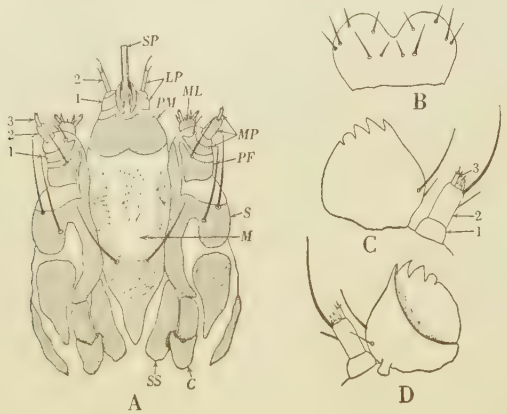
One conspicuous portion of the thorax is the thoracic plate, covering the entire dorsal area of that segment, and shining black, except the anterior margin, which is grey. The thoracic legs are black. No further additions to the setal arrangements occur after the second moult.

Prolegs. A definite tendency towards biordinal crochets, with some of intermediate length still present. Along the anterior margin of the anal prolegs from ten to nineteen crochets are present, on the other pairs the number varies from fourteen to twenty-eight.

Fifth instar.

In this, the final stage, the colour is at first similar to that of the preceding instar; on reaching maturity, however, the dark brown changes to a dull paler brown. Around the base of each seta or group of setae the chitinous plate is of a darker colour than the body. The thoracic plate is black with a grey-brown anterior margin, and the anal plate, though very dark, is more often brown than black. When fully fed the larva measures up to 1·5 cm. in length.

Head. This is shining black with setal arrangement very similar to the preceding instars. The width of the head capsule averages 1·23 mm., varying from 0·95 to 1·36 mm.



Text-fig. 7. Mouthparts of last instar larva (× 50). A, maxillae and labium, ventral view. B, labrum, dorsal view. C, right mandible and antenna, dorsal view. D, Right mandible and antenna, ventral view. *C*, cardo; *LP*, labial palp; *M*, mentum; *ML*, maxillary lobe; *MP*, maxillary palp; *PF*, palpifer; *PM*, prementum; *S*, stipes; *SP*, spinneret; *SS*, submental sclerite.

With the exception of differences in size and greater chitinization of all parts, the mouthparts of the mature larva resemble very closely those of the first instar larva.

Labrum (Text-fig. 7 B). Instead of a very acute apex to the incision midway along the anterior edge there is now a deeper and more rounded indentation. All the bristles are larger and approximate more closely to equality in size.

Mandibles (Text-fig. 7 C, D). These have lost their rectangular shape; they are now rounded laterally and relatively broader.

Maxillae (Text-fig. 7 A). Segment 2 of the maxillary palp (*MP*) has elongated until in the final instar larva it is much longer than either the basal or apical joints. The apical joint is considerably narrower in comparison with the other segments than in the first stage, and has three small papillae present on the apical extremity. From the terminal surface of the maxillary lobe (*ML*) arise two long slender papillae, another pair of papillae, two-jointed with the apical joint minute, a short central spine and from the side nearest the palp a larger bristle.

Labium (Text-fig. 7 A). The mentum is still lightly chitinized but more robust than in the early instars; the prementum is also larger.

Antennae (Text-fig. 7 C, D). A lengthening of the intermediate joint has occurred, and in the final stage larva it is more than twice as long as the basal joint. The apical joint is considerably reduced, as are the papilla and bristles carried at its extremity.

Chaetotaxy. No change in setal arrangement occurs after the third instar. It may be of interest to point out that individual larvae of the third, fourth and fifth stages seldom possess a complete set of setae. Occasionally a whole group may be missing from one segment, or only one seta from the basal plate. A seta is very often absent from the tau group on segments 1, 2 and 9 of the abdomen. If an entire group is absent there is no chitinous plate present to indicate the exact position, so this deficiency cannot be attributed to mechanical damage during the same instar or we should sometimes expect to find the base present.

Spiracles. Through each instar the relative size of individual spiracles remains constant. The prothoracic pair and those on the eighth abdominal segment are the largest and equal in size—about 0·08 mm. diameter in the mature larva. The others—on the first seven abdominal segments—are equal in size, but only a little more than half the width of the two larger pairs. A thick circular peritreme surrounds the opening of each spiracle.

Thoracic legs (Text-fig. 4 D). In all the instars the thoracic legs are identical in structure—the only difference is the degree of chitinization, each instar possessing a larger and stronger leg than the preceding one. The legs are conical, tapering from a broad coxa to the single claw. The *coxae* (*CO*) are incomplete on the outside. A large bristle is present in a median position on the inside, directly beneath sigma, and three bristles on both anterior and posterior sides, a small one at the dorsal edge towards the outer extremity, a larger bristle midway between the dorsal and ventral edges of the coxa, vertically above the lateral extremity of the trochanter, and the third on the same horizontal level but nearer the median bristle. The *trochanter* (*T*) is narrow, and the lateral extremities do not meet externally. No bristles are present. Each *femur* (*F*) is complete, with a pair of large bristles on the internal, ventral edge. *Tibiae* (*TI*) and *tarsi* (*TA*) are also entire, the former having three bristles on both anterior and posterior surfaces. These bristles become progressively larger from the outside inwards. Each tarsus has a pair of bristles near the external, ventral edge and a smaller pair on the corresponding internal edge.

Prolegs. A uniseries of biordinal crochets on the first four pairs varies in number from twenty-seven to forty-eight. Along the anterior margin of the anal prolegs are nineteen to thirty-four crochets, also biordinal. It is still possible to find the regular sequence of alternating large and small crochets broken by two of the same size.

Head capsule measurements in relation to Dyar's Law

Dyar (1890) devised a method whereby any inaccuracy in the observed number of larval moults could be detected. From studies on the larval instars of twenty-eight species of Lepidoptera, Dyar found that the width of head in successive instars followed a regular geometrical progression.

An analysis of the widths of moulted head capsules from *N. uddmanniana* L. over 2 years has given similar results to those obtained by Dyar (Tables VIII and IX).

Table VIII. *Measurements of the head capsules in all larval instars of* N. uddmanniana, 1936–7

Instar	Max. width in mm.	Min. width in mm.	Av. width in mm.	Ratio	Calculated width in mm.
I	0·23	0·19	0·213	—	—
II	0·37	0·29	0·34	1·60	0·33
III	0·64	0·46	0·54	1·59	0·51
IV	0·81	0·68	0·75	1·39	0·80
V	1·36	0·95	1·24	1·65	1·25

Mean ratio = 1·56.

Table IX. *Measurements of head capsules in all larval instars of* N. uddmanniana, 1937–8

Instar	Max. width in mm.	Min. width in mm.	Av. width in mm.	Ratio	Calculated width in mm.
I	0·23	0·20	0·216	—	—
II	0·39	0·31	0·34	1·57	0·33
III	0·61	0·46	0·53	1·56	0·51
IV	0·86	0·76	0·80	1·51	0·78
V	1·31	1·14	1·21	1·51	1·20

Mean ratio = 1·54.

The mean ratio is obtained by dividing the observed average width for each instar into that for the succeeding instar, and taking the average of the results.

Since the calculated widths agree so closely with those observed, it is most improbable that any moult has been missed. Moreover, the results for two successive generations approximate too closely to suggest any inaccuracy.

The pupa

Biology

For a period of about 3 days prior to pupation the larva does not feed. It becomes sluggish and the body colour changes to a paler brown. Slight lateral bulges appear on the meso- and metathorax, where the brown is replaced by a dirty white. These areas of paler coloration extend dorsally to the alpha-beta setae, laterally and ventrally. The head is partially retracted beneath the thoracic plate; the two posterior thoracic and the first two abdominal segments also contract. During this quiescent

period the alimentary canal is emptied and the frass excreted becomes attached to the outer surface of a small silken cocoon, which the larva spins round itself within the webbed mass of leaves. It is in this cocoon that pupation takes place.

Time of pupation.

In the field at East Malling, pupation has first been noted during the latter part of May—27. v. 36, 29. v. 37 and 20. v. 38 are earliest records—and continues for 4–5 weeks.

Table X. *Time of pupation of* N. uddmanniana *in* 1937

Date	No. of larvae	No. of pupae	Percentage pupated	Host plant
26 May	38	0	0·0	Loganberry
29	35	1	2·8	Wild blackberry
2 June	31	1	3·2	Loganberry
7	30	3	9·1	Loganberry
8	21	7	25	Wild blackberry
11	45	15	25	Wild blackberry
15	13	10	43	Loganberry
20	14	17	55	Wild blackberry
23	4	10	71	Wild blackberry
24	5	21	81	Wild blackberry
27	6*	25	89	Wild blackberry
2 July	2†	19	100	Wild blackberry

* Three larvae parasitized. † Both larvae parasitized.

Immediately pupation began in 1937, bi-weekly collections of larvae and pupae were made either from wild blackberry or loganberry as in Table X. It has been considered that each of these counts was representative of the actual proportion of larvae and pupae present in this district on the dates recorded. Text-fig. 8 shows that only a few pupate during the first week; afterwards the number changing from larva to pupa increases, then remains fairly constant until pupation is almost complete. Towards the end of the period the rate of pupation again slows down. One exception is notable, that of 8 June, for which there is no obvious explanation.

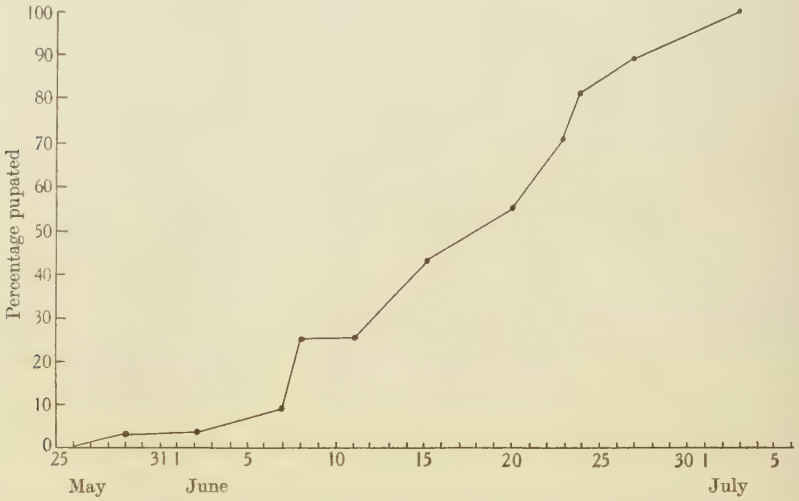
In 1937, pupation began on 29 May, and was completed by the end of June.

Duration of the pupal stage.

In the laboratory the pupal stage lasted from 12 to 16 days, with an average of 13½ days. Observations in the field gave an average of 3 weeks for this phase, the shortest period was 17 days, and the longest 28 days.

Emergence from the cocoon.

Empty pupal cases are always found protruding from the silken pupal chamber in the web as far as the tips of the wing cases. No special device for rupturing the end of the cocoon has been observed. With the aid of the dorsal spines, a wriggling motion should be sufficient to force the pupa partly outside the cocoon.



Text-fig. 8. Graph showing period and rate of pupation of *N. uddmanniana* in the field, 1937.

Emergence of the adult.

This occurs by a splitting of the pupal case along the mid-dorsal line (Text-fig. 9*X*) from the anterior edge of the prothorax to the posterior edge of the metathorax. Ventrally there is no fracture, but the antennae, together with the eyes, frons, labial palps and first coxae become free as one piece, and the mesothoracic legs tend to separate along the mid-ventral line.

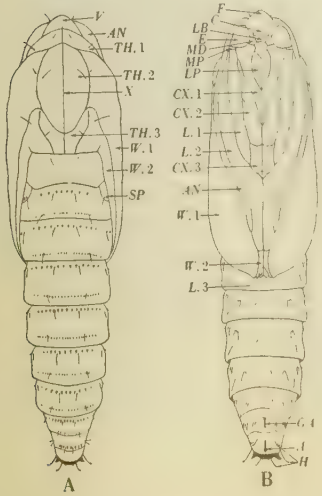
Emergence occurs both by day and night in almost equal numbers. Frequent counts by day showed no preference for any particular period.

Morphology

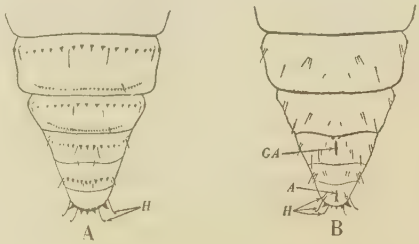
The pupa is reddish brown, and always darker on the dorsal surface, the change of colour occurring rapidly along a narrow lateral area slightly dorsal to the spiracles. Individual specimens show a marked difference in colour intensity, varying from those

with almost black wing pads and dorsal area, and dark brown ventrally, to others which are chestnut brown above and orange brown on the ventral side.

With the exception of the metathorax, which is pitted and dull, the head and thorax and appendages of these two regions are glabrous. Ventrally the wings always extend to the fourth abdominal segment, and overlap about half its length (Text-fig. 9). Mid-ventrally the anterior pair of wings (*W* 1) do not meet, and here, posterior to the extremities of the mesothoracic legs (*L* 2) may be seen a small area of the posterior pair of wings (*W* 2). Projecting beneath the wing tips, but not beyond them, are the metathoracic legs (*L* 3). The antennae (*AN*) do not quite reach to the posterior extremities of the mesothoracic legs.



Text-fig. 9.



Text-fig. 10.

Text-fig. 9. Male pupa (×8). A, dorsal view. B, ventral view. *A*, anus; *AN*, antenna; *C*, clypeus; *CX*, coxa; *E*, eye; *F*, frons; *GA*, genital aperture; *H*, hooks; *L*, leg; *LB*, labrum; *LP*, labial palp; *MD*, mandible; *MP*, maxillary palp; *SP*, spiracle; *TH*, thorax; *V*, vertex; *W*, wing; *X*, ridge along which pupa splits to allow emergence of adult.

Text-fig. 10. Female pupa. A, dorsal view. B, ventral view. *A*, anus; *GA*, genital aperture; *H*, hooks.

The abdomen has a minutely pitted surface, and is more dull than the thorax. In both sexes segments 2–7 possess, besides the normal setae, homologous to those on the mature larva, two transverse rows of backward-projecting spines on the dorsal surface. A row of large spines near the anterior margin reaches laterally to the Rho group of setae, and a row of considerably smaller spines, near the posterior margin of each segment, extends for the same distance. In both rows the spines decrease in size towards the lateral extremities. On segment 8 the anterior row is present in both sexes. The posterior row is seldom found on female pupae (Text-fig. 10), yet male

pupae nearly always have the two rows of spines, the posterior row somewhat shortened and not extending laterally beyond the beta setae. Only one row of spines is present on segment 9 in both sexes; these are of the larger size and situated transversely across the posterior half of the dorsal surface.

The distal end of the terminal segment is blunt and forms the cremaster. Four to six large spines project vertically from the dorsal edge. There are also four pairs of slender elongate bristles with recurved tips (*H*), a pair on either side of the anal pore and one hook on each side of the extreme lateral spines.

Segments 4–7 in the male and 3–6 in the female are movable. Functional spiracles (*SP*), visible externally, occur on segments 2–7 of the abdomen; those on segment 8 are vestigial. The male pupa (Text-fig. 9) possesses one genital pore (*GA*) on segment 9, the female a common opening of the bursa copulatrix and oviduct on segment 8. In both sexes the anal pore (*A*) is on the caudal margin of segment 10.

There is practically no difference in length between the two sexes. Measurements of fifty specimens of each sex gave an average length of 8·6 mm. for the male, with a variation from 7 mm. to 1·0 cm.; and the female pupa ranged from 6·3 to 9·8 mm. with an average of 8·6 mm. The female is, however, wider than the male.

Parasites

Two species of hymenopterous parasites and one dipterous parasite have been bred from the larva of *N. uddmanniana* L.

It is highly probable that the insect is kept in check to a considerable extent by the presence of an ichneumonid parasite, *Omorgus mutabilis* H.Gr. From several hundred larvae collected at East Malling during the past 3 years, the proportion found to be parasitized has not fallen below 36 % (Table XI).

Table XI. *Percentage* Notocelia uddmanniana *L. parasitized by* Omorgus mutabilis

Year	Total no. of larvae collected	No. of larvae parasitized	Percentage parasitized
1936	410	154	37·5
1937	343	123	36·0
1938	375	180	48·0

The adult parasite lays its egg in a young larva, probably the first stage. Bred female parasites, when caged with shoots containing first stage larvae, become excited, running over the leaves, and having located a larva, pierce the web and sometimes the leaf also with their ovipositor. That parasitism occurs before the spring feeding phase is certain, for larvae collected during hibernation and then isolated have several times produced parasitic cocoons.

The first indication that a larva is parasitized is partial cessation of feeding, sluggishness, and the skin becoming a darker brown and wrinkled. A few days later it becomes a grey-brown, and the parasitic larva may be observed to fill the entire body space. It breaks through the skin of its host and spins a grey, ovoid cocoon *in situ*.

The earliest parasitic cocoons are found by the middle of May, mostly from the fourth instar larvae. Other larvae of *Notocelia uddmanniana* L. reach the final instar, where the presence of the parasite may, in extreme cases, prolong the length of the fifth stage many days beyond the normal duration. After all healthy larvae have pupated, it is still possible to find occasional parasitized larvae for another week or 10 days. Occasionally the larva pupates before the parasite matures.

Omorgus mutabilis adults commence to emerge at the beginning of June and continue until the middle of July, or later.

This parasite is widespread in England. It has been bred from larvae of *Notocelia uddmanniana* received from Gloucestershire, Lincolnshire, Cambridge, Essex, Kent, Sussex and Devon.

The other larval hymenopterous parasite is an undescribed species of *Apanteles*. So far it has only been discovered in small numbers.

One specimen of a dipterous parasite (fam. Tachinidae), *Nemorilla notabilis* Mg., was bred. This parasite was undoubtedly present in the larva when collected, but did not appear until after pupation.

Host plants

Of those varieties of *Rubus* most extensively grown, only the loganberry and phenomenal berry suffer severely from attacks by this tortricid. Raspberry (*R. idaeus* L.) and the blackberries—Himalaya and Black Diamond (both probably selections from *R. procerus* P.J.M.) and Cut-Leaved (*R. laciniatus* Willd.)—are far less attractive as food plants, yet a few larvae are present on them each year. The numerous other species and hybrids of *Rubus* will all provide an adequate food supply for *Notocelia uddmanniana* L. to complete its life cycle.

Wild blackberry is definitely a favourite host plant, and is always a potential source of infestation when situated in the neighbourhood of newly planted plots of cultivated varieties of *Rubus*.

This tortricid has not been recorded from any plants belonging to a genus other than *Rubus*.

Economic importance

In England *Notocelia uddmanniana* L. is not at all uncommon on cultivated forms of *Rubus* in the main fruit-growing areas—Cambridge and Wisbech, south-east England and the Wye Valley. So far it is only in the first-named district that the larva of this moth causes serious damage, though sometimes it appears in sufficient numbers to cause anxiety to fruit-growers in the other localities. It must not, however, be overlooked that many of our present-day pests were at first noticed in small numbers, then increasing as the acreage of intensive cultivation of single crops increased.

No appreciable damage occurs from larval feeding prior to hibernation. It is from April until the larvae pupate that serious damage may be caused. The growing caterpillars feed voraciously—those on the fruiting laterals destroy the blossom buds, then move off to the young canes. Without exception, the terminal buds of webbed canes are destroyed and growth checked, to be continued later from the lateral buds, which normally would have remained dormant until the following year. The canes with much lateral growth are weaker than a single, stout cane; they also ripen less successfully in the autumn, and consequently there is a reduction in the yield of fruit the following year.

Preliminary experiments on control measures

Apart from the tedious method of hand picking, no effective control has yet been devised. From a study of the life history it seems impracticable to apply control measures to the egg stage or young larvae because both coincide with the fruiting season. There are two other possibilities:

(*a*) Applying a winter wash to the canes to kill the hibernating larvae.

(*b*) Spraying or dusting after the larvae emerge from hibernation.

Canes containing hibernating larvae were dipped in a 10% tar distillate wash, but no control was obtained. It seems that the cocoon within which the larva spends the winter months is impervious to a tar wash.

In April 1937, when the first larvae began to emerge from hibernation, a spray containing 4 lb. of lead arsenate paste to 100 gal. of water was applied to a small plot of phenomenal berries. Plant growth was so rapid that new leaves with no spray residue were observed 2 days

after spraying. It was to these leaves that most of the larvae migrated. No difference between sprayed and control plants could be detected.

A similar experiment was carried out using 2 lb. of a proprietary derris extract and 6 lb. of soft soap per 100 gal. It was hoped that the derris on the older leaves would have a residual, contact, toxic action even if the larvae did not feed on the leaves. With one application when the first larva was seen and another 10 days later no control was obtained.

An atomized pyrethrum spray was used experimentally as a possible means of control in 1938. The spraying was done with a hand atomizer, using a pyrethrum extract diluted to 0·5 % pyrethrins content in a base of highly refined white oil of the kerosene type (viscosity 30° Saybolt at 100° F. and 99 % insoluble in concentrated sulphuric acid). On 4 April, before all the larvae had left their winter cocoons, twenty plants were sprayed. A count the following day showed that, out of forty-two larvae, forty were dead; one died later and the other survived. A field trial was immediately arranged to test this spray further. Owing to various difficulties it was not completed until 6 May. By this time all the larvae had spun large webs, and penetration by the spray was undoubtedly less than at an earlier date. The experimental spray, reduced to 0·25 % pyrethrins content, gave a control of only 6·5 %, and a proprietary spray[1] of unknown composition reduced the infestation by 3·7 %, each on plots of one-third of an acre.

The atomized pyrethrum is to be used again in 1939, when the application will be made at a much earlier date, before the larvae have had time to spin large webs.

Summary

A detailed study of the morphology and biology of all stages in the life cycle of *Notocelia uddmanniana* L. has been made over a period of 2½ years.

This tortricid is a pest of loganberry in certain fruit-growing areas.

The widths of the head capsules in all larval instars have been measured and found to follow Dyar's Law.

In the East Malling district an ichneumonid, *Omorgus mutabilis* H.Gr., parasitizes 35–50 % of the larvae. A few specimens of an undescribed species of *Apanteles* have been bred from larvae, and also a tachinid fly, *Nemorilla notabilis* Mg.

Preliminary control measures have given poor results.

[1] The writer is indebted to Mr J. V. Lewis of Messrs Strawson's Ltd., who kindly loaned the atomizing unit and provided the proprietary spray.

The writer wishes to acknowledge his indebtedness to Dr A. M. Massee, who has supervised this work from the earliest stages, also to Mr J. E. Collin and Mr J. F. Perkins, who kindly identified the dipterous and hymenopterous parasites respectively, and to the Advisory Entomologists and fruit-growers who have helped with the collection of material. Also to Dr C. B. Williams who placed at the author's disposal his unpublished records of captures of *Notocelia uddmanniana* in the Rothamsted light trap.

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EXPLANATION OF PLATE XL

Fig. 1. *Notocelia uddmanniana* L. Female ($\times 2\frac{1}{2}$).

Fig. 2. Eggs of *N. uddmanniana* L. laid by caged adults ($\times 8$).

Fig. 3. Damage to tip of cultivated blackberry cane by larva of *N. uddmanniana* L.

(*Received* 15 *March* 1939)

Fig. 3.

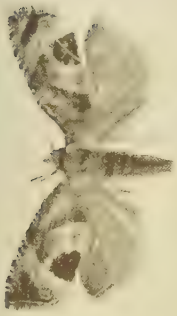

Fig. 1.

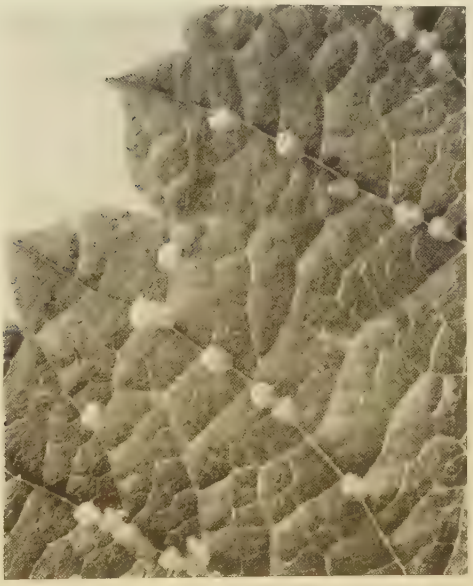

Fig. 2.

DICKER.—The Morphology and Biology of the Bramble Shoot-Webber, *Notocelia uddmanniana* L. (Tortricidae) (pp. 710–738)

FURTHER OBSERVATIONS ON *ANGUILLULINA DIPSACI* IN RHUBARB

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(With Plate XLI)

CONTENTS

Introduction

Johnson (1936) referred to the occurrence of *Anguillulina dipsaci* in dormant rhubarb root stocks in Yorkshire. It was stated that eelworm infection is associated with a diseased condition the symptoms of which are indistinguishable from those of crown rot described by Millard (1924) who reproduced the disease experimentally by inoculation with *Bacterium rhaponticum* isolated from diseased rhubarb. If it is accepted that in the field there are two separate diseases, namely bacterial crown rot and stem eelworm disease, observations made on material from a large number of holdings show that *A. dipsaci* infection is very widespread. A root stock showing typical disease symptoms similar to those described by Millard is likely to yield *A. dipsaci* in large numbers on laboratory examination. It is the almost invariable occurrence of the eelworm on rhubarb holdings in Yorkshire which cannot fail to suggest strongly that *A. dipsaci* is a most serious primary cause of disease in this host as it is in so many other cultivated plants. Reference has already been made (Johnson, 1936) to the similarity between stem eelworm disease in rhubarb and the same

disease in mangolds, as described by Goodey (1929). More recently Roebuck & Hull (1937) have described a crown canker of sugar beet associated with eelworm infection, and Walton (1936) has described a crown canker of parsnips attributed to *A. dipsaci*. White, in a series of notes (1933, 1934, 1935, 1936), reports on investigations on disease in rhubarb, in which he isolated a number of fungi and bacteria from diseased rhubarb and claimed to have isolated an organism comparable with *B. rhaponticum* as described by Millard. Negative results attended his attempts to reproduce the disease by inoculation. He concludes that "the negative results of this investigation, so far, are difficult to interpret on the view that the complex of symptoms usually termed crown rot is solely a disease of bacterial origin". White, like Millard, makes no mention of the presence or absence of *A. dipsaci* in his material obtained in the fields.

It is interesting to note that White refers to the occurrence of brown pockets in the centre of rhubarb roots, these pockets being "cut off by the formation of a skin" and being free from organisms. Millard also referred to pockets, enclosed by a skin, some of which he regards as lesions of bacterial crown rot, and he mentions the natural hollowing, remarking that it is found frequently in healthy plants, especially of the variety Victoria. The present writer has often seen these pockets, especially in the field when the roots are being split for propagation, and it is possible that the sterile brown areas described by White are comparable with these brown pockets which appear to be a prelude to natural hollowing. Plate XLI, fig. 1 shows a portion of a young sett raised from seed with a series of brown pockets in various stages of development, and in this case they would appear to be no more than a series of differentiating internodal hollows of the base of a flowering stem, which had not yet elongated. It is therefore evident that all rotting symptoms, or brown tissue, are not of necessity crown rot due either to bacterial or eelworm infection, and, in America, Beach (1923) has described a crown rot of rhubarb caused by *Phytophthora cactorum*.

The present writer's observations so far suggest that where active *A. dipsaci* infection is present in diseased rhubarb roots, there is not the tendency for the formation of a skin of suberized tissue completely to cut off the decayed lesion.

The incidence of *A. dipsaci* in association with disease of rhubarb is not confined to Yorkshire, for Mr W. E. H. Hodson, Advisory Entomologist in the Southern Advisory Province, has kindly informed the writer that he has recorded stem eelworm injury to rhubarb in his area. The

writer has also found *A. dipsaci* in diseased roots sent from Scotland and Middlesex. Johnson (1936) referred mainly to eelworm infection of dormant root stocks. The results of continued field observations and certain experiments on the bionomics of the eelworm are summarized in the present paper.

FIELD OBSERVATIONS

Continued opportunities of examining rhubarb plants from many holdings in Yorkshire confirmed previous observations, and strengthened the view that it is difficult to find a holding which is completely free from *A. dipsaci* infection of the rhubarb.

The ease with which infection can be carried in infected setts from field to field and from holding to holding is perhaps the chief source of trouble, and the importance of alternative cropping with the right type of crop, in conjunction with general hygienic measures, has been stressed (Johnson, 1936). Recently, cases have been encountered which serve to emphasize these points still further. On one farm, for example, the disease accompanied by very heavy eelworm infection was widespread in the three varieties, Victoria, Prince Albert and Dawes Champion. This state of affairs might be partly attributed to the grower's ignorance of the nature of the disease, and therefore to his failure to take proper steps in time to check it, but the rotation practised was rhubarb, potatoes, oats, seeds, and the capacity of the rhubarb strain of *A. dipsaci* readily to infect oats has been recorded by the writer. These preliminary observations have been abundantly confirmed in repeated experiments. On a second farm some six thousand roots in one field were badly diseased, heavy eelworm infection was present and the rotation had been rhubarb, oats, peas, rhubarb. Here again the alternative cropping might be criticized as likely to foster eelworm infection in the field. The farmer reported that the oats grown 2 years previously were badly "segged", and although this condition may have been due to frit-fly injury, it is not impossible that the oats were suffering from stem eelworm disease. Under experimental conditions the rhubarb strain of *A. dipsaci* has also been found to be capable of producing persistent and very injurious infection of peas.

Field inspections made during the growing period have furnished information on the extent to which the foliage may be infected by the eelworm, and diseased setts have also been taken from the field and grown in pots for observation during the summer. Millard, in his description of bacterial crown rot, states that the bases of the leaf stalks

may be swollen and that "such stalks show a brown discoloration at their junction with the crown". He also refers to the discoloration of the leaves and notes that the foliage of diseased plants often turns a characteristic puce colour. The present writer's observations have shown that rhubarb plants, suffering badly from crown rot associated with infection by *A. dipsaci*, may exhibit precisely similar symptoms in the foliage. On one of the holdings referred to earlier, where a field of rhubarb was very seriously affected by disease, large numbers of plants had reddish coloured foliage in May and on pulling at the stalks of such plants a portion of the diseased crown came away with the stalks. Laboratory examination revealed typical symptoms of both bacterial crown rot, as described by Millard, and stem eelworm disease, as noted by the writer. Thus, extensive rotting was present in the crown region extending often into the fang roots; brown discoloration sometimes, but not always, associated with swelling, occurred at the base of the leaf stalks and lesions extending into lateral buds from the diseased tissue below were observed. In all these lesions heavy infection by *A. dipsaci* occurred. These findings are similar to others noted in the field and in plants transplanted from the field into pots. The similarity therefore between Millard's description of bacterial crown rot and the symptoms of stem eelworm disease in rhubarb render it impossible to distinguish between the two diseases in the field. A disease in which *A. dipsaci* is involved is likely to be more difficult to control than one for which a bacterium alone is responsible, and it is essential, therefore, that the presence or absence of stem eelworm should always be determined in diseased rhubarb, for the result of such an examination will determine the steps to be taken to eradicate and prevent the disease. The question of the choice of suitable alternative crops is also much more important in relation to the stem eelworm, since certain crops may be potential carriers of the rhubarb strain, whereas this consideration would not be involved in the case of the bacterial disease.

Experimental

The purpose of the experiments to be described has been to induce eelworm infection of the rhubarb plant, to follow the course of the infection and accompanying symptoms, and to investigate the possibilities of transference of the rhubarb strain of *A. dipsaci* to other hosts, in particular to some of the crops commonly used in the rotation on rhubarb farms in Yorkshire. Experiments have been made both out of doors and in the cold glasshouse. In the indoor experiments plants were

grown directly from seed sown in pots containing eelworm free soil and the infection was introduced usually by the addition of dry infective plant material. Out of doors various methods have been used, namely ordinary pots sunk in the soil, glazed pots resting on the surface, and zinc cylinders sunk in the soil, as described by Hodson (1931). Infective material was added either before sowing the seeds of the host or after transplanted seedlings raised in eelworm free soil had become properly established. In all individual experiments each type of infection was carried out at least in duplicate with an adequate number of controls. Since infection experiments in ordinary pots are not always satisfactory, the cylinder method was used as a check on some of the pot experiments. In the following pages the experiments are summarized according to the host plant used.

Rhubarb

During the past 3 years a considerable number of experiments has been carried out and repeated using rhubarb as the host. In the first type of experiment rhubarb seed of the variety Victoria was sown in early spring in eelworm free soil to which in one series dried rhubarb chips had been added, the other being retained without infection as controls. Both ordinary pots and large glazed pots with a single outlet which could be corked when necessary were employed. The latter type of pot proved to be very satisfactory, possibly because the watering of the soil is more easily controlled than in the ordinary plant pots which may partially dry out quickly. The pots were kept in the cool glasshouse until late spring when they were moved outside. In the infected series, seedlings quickly became infected. Three weeks after sowing, for example, some seedlings exhibited distinct swellings of the hypocotyl below the cotyledons. Invasion of the cotyledons occurred, accompanied by distortion, and as the true leaves developed the infection passed into the petioles and leaves, resulting in very marked distortion of the foliage. A characteristic feature of eelworm infection of such leaves is a somewhat swollen corrugated appearance of both the petiole and infected veins. Infected leaves wilted, the base of the petioles turned brown and rotten, splitting occurred and the leaves eventually fell. A proportion of the seedlings died very early. As the season progressed all stages of what might be termed crown rot in miniature were observed in the young plants. Thus, in some, the top of the plant was easily detached and severe rotting, associated with heavy eelworm infection of the crown region, was revealed. Other plants exhibited earlier stages in the disease,

e.g. the plant shown in Pl. XLI, fig. 2, in which a cavity is just beginning to form beneath the crown. Heavy eelworm infection occurs in such plants all around the cavity and extends downwards into the tissue of the root, the extent of infection being perceptible in a sliced specimen by its light brown colour contrasting with the almost white healthy tissue outside it. All stages of the disease were obtained leading up to death of the seedling.

In a second type of experiment rhubarb seedlings were grown in cylinders sunk in the soil, the seedlings being transplanted into the cylinders in July. After the plants had fully recovered, dried infective rhubarb chips were mixed into the top soil of the infected series. Within 15 days infection was established in the foliage, several leaves were wilting, and some showed discoloured patches. These symptoms were associated with eelworm infection at the base of the leaf stalks. Subsequent examinations revealed the progress of infection which commenced in the leaf stalks and gradually passed up into the leaf itself by way of the main veins. Swelling of the base of the stalk sometimes, but not invariably, occurred, for in many cases the leaf stalk quickly rotted and split. Infected leaf stalks were often soft, and on being split open the interior was found to be discoloured and in many cases became dry and powdery; very large numbers of *A. dipsaci* in all stages could be recovered from these sites. Leaf infection was again characterized by the swollen, often discoloured and corrugated veins which internally revealed discoloration. Puckering of the leaf, especially at the junction of the main veins, frequently occurred. The eelworm infection had passed from the base of the leaf stalk to the ultimate tip of the leaf within 3 weeks after the introduction of infested material. Whole plants were lifted at intervals to ascertain the presence or absence of infection in the crown, but it was not until some 12 months after planting that there was any sign of disease in the setts. At this time it was clear that eelworm infection had invaded the root stock below the region of the lateral buds and rot extended from the outside of the plant to the tissue below the crown. It should be borne in mind that as the leaves and their stalks became heavily infected and wilted, they were removed and by this means a very considerable amount of potential infection was removed from the infected cylinders. All control plants remained free from infection.

During 1936 seedlings from the same batch as those used in the cylinder experiments were transplanted to a plot until all the foliage had died down, and in early November seven plants were transferred to

seven pots sunk in the soil. Three pots were infected by the addition of dry infective material; the other four were kept as controls and remained free from infection throughout the course of the experiment. In the infected pots the plants showed obvious signs of infection by mid-May the following year, the foliage developing typical symptoms; red spots frequently occurred on the leaves in association with infection. The plants were lifted on 5 December 1937, i.e. about 13 months after planting, and all showed signs of disease in the root stock. Two setts were not extensively diseased but well-defined lesions of brownish rot accompanied by eelworm infection occurred in the lateral aspect of each root and extended towards the crown. Certain lateral buds and the bases of fang roots near the main crown lesion were also affected. The third plant had reached a very advanced stage of the disease, the main crown having been destroyed and the very extensive rot had passed into some of the fang roots. The infection by *A. dipsaci* was extremely heavy. Pl. XLI, fig. 3, shows this plant cut longitudinally, and the symptoms recall those described and illustrated by Johnson (1936) of the disease in the field, developed as a result of natural infection. It is also clear that these experimentally produced symptoms are comparable with those typical of bacterial crown rot, as described by Millard. Eelworm infection has, however, not yet been observed in the leaves themselves in the field, but only in the bases of the leaf stalks. The reason may be that in most cases in the field the infection in the leaf stalk is preceded by well-established infection in the crown, and leaf stalk infection develops directly from that in the crown. In the infection experiments quoted, on the other hand, where young healthy seedlings are subjected to eelworm infection, the young leaf stalks become infected first and the infection advances rapidly in the leaves and finally invades the crown and root stock.

Oats

The preliminary observations that the rhubarb strain passed freely to oats in pot experiments using black winter oats have been confirmed. Pot and cylinder experiments using Victory oats also gave similar results. In the cylinder tests Victory oats sown in mid-March showed obvious signs of infection by mid-April. Typical severe "tulip-root" symptoms subsequently supervened in the infected series, whereas the control plants grew normally.

The fact that the rhubarb strain and an oat strain of *A. dipsaci* are reciprocally infective was reported by the writer, and this conclusion

has been confirmed in more recent experiments using infective oat material and rhubarb seedlings.

Peas

The pea plant was selected for trial because this crop is commonly grown in Yorkshire as one of the crops alternative to rhubarb, and on some farms where this crop is frequently sown the extent of rhubarb disease associated with eelworm infestation is very considerable.

One of the best growers in Yorkshire commonly makes use of the pea crop as one of the alternatives to rhubarb. In the past he has had no obvious signs of disease in his holding, but during the last 3 years, in spite of the fact that he did not introduce rhubarb stocks from outside, the disease has gradually gained ground, and is now sufficiently evident to cause serious concern. The writer has not, however, had opportunity to examine carefully pea crops on such farms for eelworm infection. Larval eelworms have been obtained from pea seedlings taken at random in the field but, although these eelworms possessed a buccal spine, they were not at a stage at which their identity could be established.

Controlled infection experiments were conducted first in pots in the cool glasshouse. Pea seedlings were raised in pots in soil to which eelworm-infested rhubarb material had previously been added, and a similar series were grown without the addition of eelworm infection. All the plants in the infected pots became heavily infested, and the infestation persisted throughout the life of the plants. The control plants remained healthy.

The experiment was repeated out of doors in cylinders and, as in the pot experiments, a persistent eelworm infestation occurred in the infected series associated with definite disease symptoms. Within 1 month after sowing in late March, all stages of the eelworm were recovered from the base of infected plants. Subsequent examinations revealed the passage of infection up the plants. The lower leaves were quickly invaded in both petiole and leaflet, and during the course of the season these lower leaves died prematurely. The basal portion of the stem eventually became discoloured and a similar brownish discoloration of the pith was associated with heavy eelworm infection. The control plants progressed normally and at the time of pod formation the surviving infected plants were never more than half the length of the controls. Some of the infected plants were so dwarfed that 3 months after sowing they had attained a height of only 3 in.

Mangolds

It was decided to test mangolds not only because this crop is grown on some rhubarb farms but also on account of the great similarity in the symptoms of eelworm disease in mangolds and crown rot of rhubarb. Two series of cylinder experiments were conducted; the first in April and the second in June. The results were similar in both cases. Fourteen days after sowing, the first signs of infection were noticed in a few of the seedlings in the infected cylinders. The early symptoms were the usual discoloured spots on the cotyledons continuous with a discoloured lesion extending along the petiole, and dissection of such parts disclosed *A. dipsaci* in numbers. One cotyledon only showed these symptoms. The progress of the infection produced distortion of the cotyledon, the infected side being shorter than the other, and twisting of the leaf finally occurred.

Pl. XLI, fig. 4, illustrates the kind of distortion which occurred. From this point the course of infection produced one of two effects on the plant. In some cases the seedlings failed to produce true leaves and such plants died, the hypocotyl region being heavily infected and severe rotting ensuing. Seedlings which produced true leaves showed some infection of the lowermost leaves, the petiole being invaded, resulting in some distortion of the leaf. The infection, however, failed to persist from this point onwards, and the surviving plants grew normally and no subsequent eelworm infection was observed.

Cabbage

It has already been stated that although the brassica crop is often the only crop alternative to rhubarb in the rotation, many growers are of opinion that a rest of only 1 year under a brassica crop is sufficient to effect a significant check on the disease. Examination of various brassicas taken in the field has so far yielded no signs of infection by *A. dipsaci*.

Infection experiments have been carried out in glazed pots in the cold greenhouse and out of doors, using cylinders. The results of these tests were similar to those obtained from the mangold experiments previously described. Control plants in both series remained free from infection, but seedlings in the infected pots and cylinders readily became infected. Thus, within 3 weeks after sowing in the cylinders, slight eelworm infection was observed just below soil surface. Twenty-six days after sowing, eelworm infection was apparent in the cotyledons in the

form of pale lesions in one cotyledon continuous with a lesion in the petiole. Distortion of infected cotyledons occurred as with mangolds, and growth of the petiole was frequently very much reduced. In some cases the stems of the infected plants were swollen, giving a corrugated appearance, and in some there was a distinct bulbous swelling at the top of the stem. All stages of the eelworm were recovered from infected parts. Later examinations showed that although in a few cases infection occurred in the base of true leaf stalks, the infection quickly died out and it seemed safe to assume that under the conditions of the experiment the rhubarb strain of *A. dipsaci* is unable to produce a persistent infection in cabbage.

Weeds

During the course of these experiments the opportunity was taken of examining for eelworm infection adventitious weeds which appeared in the pots and cylinders. Chickweed (*Stellaria media*) occurred freely and regular examinations made throughout the growing season proved that the rhubarb strain of *A. dipsaci* transfers freely to chickweed. In groundsel (*Senecio vulgaris*) and plantain (*Plantago lanceolata*), the latter being transplanted into infected cylinders and pots, no infection could be detected. A few *Rumex* seedlings, which were probably *R. crispus* and *R. acetosella*, became infected, but so far it is not known to what extent the rhubarb strain of *A. dipsaci* is capable of maintaining itself permanently on these weeds.

It is clear that the strain of *A. dipsaci* occurring in rhubarb is a generalized type in its feeding habits, which emphasizes the need for precise information as to what cultivated hosts are likely to carry infection in order that the grower may make a suitable choice of crops to use as alternatives to rhubarb in the rotation.

Summary

Continued field observations on disease in rhubarb accompanied by infection by *Anguillulina dipsaci* are recorded. The symptoms, both in the foliage and in the root stock, are similar to those described for bacterial crown rot by Millard.

In infection experiments using the rhubarb strain of *A. dipsaci* symptoms of disease similar to those found in the field were reproduced in rhubarb seedlings and setts. The course of eelworm infection in the young leaves and in the crown in these experiments is recorded.

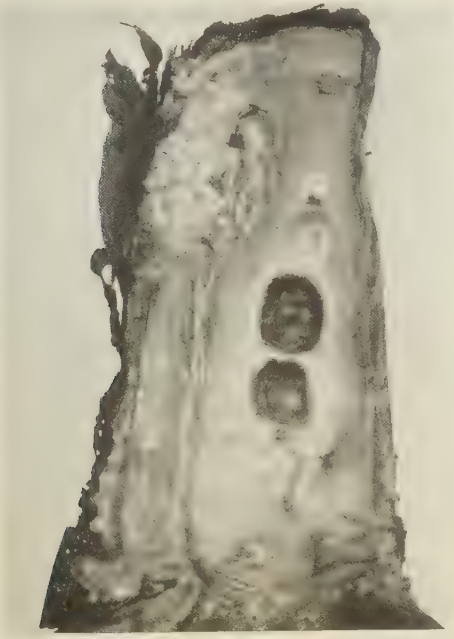

Fig. 1.

Fig. 2.

Fig. 3

Fig. 4.

JOHNSON.—Further Observations on *Anguillulina dipsaci* in Rhubarb (pp. 739–749)

Host transference experiments have shown that the rhubarb strain of *A. dipsaci* is not rigidly restricted in its feeding habits for it is capable of producing persistent infection in oats, pea and chickweed. It is also capable of producing only temporary infection in cabbage and mangold. Infection is also recorded in *Rumex* spp. but insufficient evidence is available as to its persistence on these hosts.

Incidence of *A. dipsaci* is recorded in rhubarb from Scotland and southern England in addition to Yorkshire.

The writer desires to thank his colleague Mr C. E. Hudson for his continued interest and assistance, and Mr J. Manby and Mr W. R. Wilson for the photographs.

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EXPLANATION OF PLATE XLI

Fig. 1. Crown of rhubarb plant sliced to show non-pathological internodal hollowings developing in base of flowering stem.

Fig. 2. Rhubarb seedling sliced to show formation of cavity below crown resulting from *A. dipsaci* infection.

Fig. 3. Rhubarb sett grown in eelworm infected soil showing advanced symptoms of crown rot accompanied by heavy eelworm infection.

Fig. 4. Mangold seedlings infected by the rhubarb strain of *A. dipsaci*. Note distortion of cotyledon.

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THE EFFECT OF CLIMATIC EXPOSURE ON TEXTILE FIBRES AND FABRICS

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(With Plate XLII and 3 Text-figures)

CONTENTS

1. Introduction

Prior to 1930 the relevant technical literature contained occasional references to observations made on the "mildewing" of textile fabrics, and several writers on the subject expressed views both on the causes of "mildewing" and the treatment to which fabrics should be subjected

for protection against it. Such views, however, were seldom well supported by experimental evidence. A review of this literature was given by Thaysen & Bunker in 1930.

Because of the lack of well-authenticated information and also because of the practical importance of the subject, the writers undertook, with the approval of the Department of Scientific and Industrial Research, a comprehensive investigation of the subject. It was decided to expose to a range of climatic conditions samples of fabrics of a wide and varied nature so that evidence might be obtained regarding both the participation of micro-organisms in the "mildewing" of fabrics, and the conditions under which "mildewing" and other deterioration of fabrics would occur. Subsidiary objects were to ascertain the types of organisms which are destructive to textiles, a characterization of the nature of the damage caused, and an elaboration of methods by which the damage could be avoided or minimized.

2. Methods

For this purpose a range of fabrics was exposed at one or more of the following stations, which were selected with a view to including as wide a range of climatic conditions in the test as possible.

Australia (Darwin), (Broken Hill South), (Adelaide)
Ceylon (Peradeniya)
Cyprus (Nicosia)
England (Holton Heath, Dorset)
Federated Malay States (Kuala Lumpur)
Kenya (Nairobi)
South Africa (Pretoria)
Trinidad (St Augustine)
Ceylon (Colombo Harbour, for marine exposure)

The materials exposed comprised three types of cotton fabric, in each case with its corresponding yarn sample; two types of linen canvas; one wool fabric; one hemp fabric; two silk fabrics, with and without gum discharged; three rayon fabrics; and one jute fabric. The rayon fabrics had been prepared, in one case by the cuprammonium process, in another by the viscose process; the third type was a cellulose acetate fabric. Detailed analyses were on record of all the various fabrics, and it was possible, therefore, to estimate to what extent a change in the fabric during exposure might have been influenced by peculiarities in the fabric itself.

Through the collaboration of scientific workers at the stations mentioned it was possible to expose the fabrics under all conditions likely to be met with during the normal use of fabrics. The actual procedure for the exposure was communicated to the supervisors with the samples of fabrics sent to them from the writers' laboratory. Prior to dispatch the fabric samples, measuring 10×7 in., had been placed in stoppered glass tubes and subjected to a careful process of heat sterilization in order to

eliminate all harmful micro-organisms which might have been contained in the fabrics. Suitable aluminium frames for holding the fabrics during exposure were also supplied.

The following methods of exposure were adopted at all stations (except at Colombo Harbour, where the fabrics, suspended in their frames, were immersed in the sea at the guide pier to the harbour slip-way, 3 ft. below the surface and at such distances apart that they were unable to foul each other).

Two frames of each fabric (except of hemp, of which only one was taken) were used for each series of experiments. In one series the samples were buried vertically in open, not grass-covered, soil. In another series two frames of each fabric were hung in a place protected from the direct rays of the sun, but not sheltered from rain, and in a third series two frames of each fabric were hung in direct sunlight.

The various samples were examined periodically during exposure and the experiments were continued for a period not exceeding one year. Where the periodical examination revealed that some of the samples had deteriorated markedly before the year had elapsed they were removed at once, irrespective of time of exposure. In removing the samples handling was avoided as far as possible. In the case of the samples which had been buried, any loose soil adhering to the fabric was removed by gentle shaking and then the sample was immediately placed in its proper tube without further treatment. The whole operation was carried out in a sheltered place. In the case of samples which had been badly damaged, care was taken to avoid tearing. Any samples which had deteriorated more rapidly than others, and had in consequence to be returned to their tubes earlier, were kept in as cool a place as possible so that their further deterioration would proceed slowly, if at all.

As it was thought desirable to compare the microflora of the soils to which the fabrics had been exposed with that which might be found on the fabrics after exposure, provision was made for soil samples to be forwarded in suitable containers to the writers' laboratory together with the exposed fabrics.

The samples exposed in the sea at Colombo Harbour were examined at least once a fortnight during exposure and, as soon as serious rotting was observed, they were returned to their respective tubes and dispatched to England.

At the home station, in Dorset, a sufficient number of fabric samples over and above the standard number was exposed to make it possible to carry out analyses at frequent intervals.

At practically all the stations abroad the rate at which the fabrics were destroyed in the soil was much greater than had been expected and, not infrequently, most of the fabrics had disappeared when the samples were removed for final examination. For this reason further samples were sent to the stations in the Malay States and in Ceylon. On return to the laboratory the various colonial samples were stored on ice until they could be examined. The home samples were analysed immediately on their removal from the exposure ground.

Since the purpose of the investigation was to ascertain the part played by micro-organisms in damage suffered by the exposed fabrics it was desirable to use as many different culture media and as varied a technique as possible in order to isolate all the organisms which could be made to grow artificially.

In addition to the ordinary bacteriological media, therefore, such as broth agar, broth gelatine and wort agar, various others containing cellulose and hemicelluloses

as carbohydrates were employed in the process of analysis. The cellulose medium contained the following additional substances:

Dipotassium hydrogen phosphate 0·1 %
Magnesium sulphate (crystalline) 0·1 %
Sodium chloride 0·1 %
Peptone 0·2 %
Calcium carbonate 2·0 %
Tap water 97·5 %

In the analyses both aerobic and anaerobic mesophilic and thermophilic organisms were searched for and series of inoculated media were incubated both at 30 and 65° C., in each case in the presence and the absence of air.

For the detection of wool-decomposing micro-organisms a suitable part of the exposed wool fabric was placed in containers with a piece of sterile wool and a convenient quantity of food solution of the composition referred to above, as used for the isolation of cellulose-decomposing organisms.

3. RÉSUMÉ OF EXPERIMENTAL OBSERVATIONS

The brief account given above of the plan of the investigation and of the technique adopted will have made it clear that the examination of the various exposed fabrics involved a very large number of bacteriological analyses of which it would be impossible to give a detailed account in these pages. In this communication, however, a résumé will be given of the observations made in so far as they have a bearing on the problem of the possible participation of micro-organisms in the deterioration of fabrics exposed to climatic conditions. For the purpose in view these conditions have been divided into five different groups. The first comprises those conditions which cause the complete and continuous saturation of the fabric with water. Such conditions were represented in the tests by the exposure trials in Colombo Harbour. The second represents the exposure to permanently damp, but not waterlogged conditions, such as are met with in most soil exposures and in shade exposures at certain tropical stations. In the shade exposure trials, the test fabrics were hung in the air in their respective frames and were shielded from all direct sunlight, but not from rain. Though it was assumed that these two sets of conditions would be similar as regards moisture conditions it was appreciated that the similarity did not take into account the important influence which might be exercised by differences in microflora in the two habitats.

The third condition involved exposure of the fabrics to occasional wetting with subsequent drying, as represented by shade exposure of the fabrics in temperate climates; the fourth to shade exposure in arid

climates where the moisture content of the exposed fabrics would be permanently below the minimum at which micro-organisms could be active. Finally, the fifth group comprised exposure of the test fabrics in the sun, some at high humidities, as in tropical climates; some in the sun in temperate climates, and others in the sun in arid climates. This group was included for the specific purpose of studying the influence of sunlight on exposed fabrics.

4. The part played by micro-organisms in the damage suffered by textiles on climatic exposure

(*a*) *Exposure of fabrics to waterlogged aerobic conditions*

In the test three types of cotton fabric were exposed with two samples of linen canvas and one each of hemp and wool. One striking feature of the exposure was the unexpectedly rapid deterioration of the materials. The officer supervising the exposure, which was carried out in Colombo Harbour, remarked on this point in his report. His first examination of the various fabrics was made 15 days after their immersion, when it was found that two of them, the Egyptian cotton and the wool, had partly broken away from the frames supporting them. Subsequent daily examinations indicated a rapidly progressing destruction, so rapid in fact that a fabric (American cotton) which on one day appeared sound, on the following day had collapsed in its frame. Only in the case of one of the flax canvases, a very thick and closely woven fabric, was it necessary to continue the exposure for 50 days before a complete destruction of this material had occurred; in most cases the fabrics were destroyed in less than 30 days.

Compared with the various cellulosic fabrics the woollen material decayed particularly rapidly in spite of being considerably heavier than the most closely woven cotton fabric. This observation does not agree with that of Dorée (1920) who found wool more resistant than cotton when exposed in sea water in a temperate climate.

In analysing the results obtained it must be admitted at once that the expected direct evidence of the participation of micro-organisms in the deterioration of fabrics under waterlogged conditions could not be secured from the bacteriological analyses of the exposed samples. The anticipated presence of overwhelming numbers of cellulose-decomposing micro-organisms on the cellulose fabrics and of wool-decomposing types on the wool was greatly obscured by the presence on the damaged fabrics not only of a varied secondary microflora which it is exceedingly

improbable could have taken a direct part in the deterioration of the fabrics, but also of types expected to be exclusive to cellulose fabrics on the wool sample, and of wool-decomposing types on the exposed cellulosic fabrics.

It was necessary, therefore, to have recourse to a more indirect method for settling the nature of the agency which had caused the deterioration. For this purpose the microscopic methods devised by Fleming & Thaysen (1920) and by Bright (1926) for cellulose fibres were the only methods available. The claim made for these tests that it is possible by them to distinguish microscopically between microbiological damage on the one hand and chemical, physical and mechanical on the other, has been confirmed for cellulosic fibres by Searle (1924) and Burns (1927). By these microscopical examinations no difficulty was found in ascertaining that the three cotton fabrics had deteriorated extensively owing to attack by micro-organisms. In all the cases examined the swelling test of Fleming & Thaysen and the Congo red test of Bright showed that practically every hair in the deteriorated fabrics had the appearance under the microscope which is characteristic of microbiological destruction. In no single instance was action of a non-biological agency detected.

On the evidence of the microscopic tests the conclusion was drawn, therefore, that the destruction of all the cellulose fabrics exposed in the sea at Colombo under waterlogged conditions was due solely to microbiological activity, thus confirming Dorée's (1920) laboratory tests by large-scale exposure trials.

In the case of the exposed wool sample a microscopic examination was much less conclusive. Here the expected accumulation of epithelial scales was missing. Such accumulation might have indicated activity by proteolytic micro-organisms. In its stead was found an aggregation of fragments of wool hairs of a much smaller diameter than that of normal hairs, an appearance which indicated the elimination of the scales and a subsequent exposure of the core of the hairs. There is, at present, no evidence to confirm that such changes in wool are characteristic of microbiological activity, and since the evidence obtainable through bacteriological analyses also failed to yield conclusive evidence it is impossible at present, and from the exposure tests in Colombo Harbour, to arrive at a definite conclusion as to the agency responsible for the destruction of the immersed wool fabric.

Though the direct bacteriological analyses of the various sea-exposed fabrics failed to give an unequivocal answer to the question of the nature

of the agency responsible for the decay, they were not without interest in other respects. Thus they confirmed a widely held view (Thaysen & Bunker, 1927) that fungi take no active part in the destruction of organic substances under waterlogged conditions, even where oxygen has free access to the substances. Among the varied microflora of the sea-exposed fabrics comparatively few types of fungi occurred and those found were never present in appreciable numbers.

Even more striking was the absence of actinomycetes which were found in only small numbers represented by some six different types as against 23 types of fungi and 125 types of bacteria and cocci.

In no case were algae detected on the exposed fabrics, though a species was isolated from the harbour mud.

Of the bacteria and cocci found, by far the largest number were types of what may be described as a "secondary microflora". Nearly all of them grew well on ordinary bacteriological media; they failed to attack cellulose, either lignified or unlignified, while some of them were able to decompose wool. Of types capable of decomposing cellulose in the form of filter paper, some grew most readily under mesophilic aerobic conditions, others at thermophilic aerobic temperatures. Others again grew best under anaerobic conditions either at 30–37° or at 60° C.

Of special interest was a group of spore-forming rods of the *Clostridium* type which was frequently met with on the various cellulosic fabrics; they grew particularly well in media containing hemicelluloses such as xylan. This particular carbohydrate was broken down by them with the evolution of gas and the production of undetermined alcoholic compounds. It was noticeable that these hemicellulose-decomposing types were much more numerous on the exposed fabrics than in the mud from the harbour.

It was mentioned above that the supervisor of the exposure tests at Colombo Harbour had remarked on the rapid rate at which the exposed fabrics decomposed. When similar samples of cotton materials were exposed in the sea at the station in England the rate of destruction was undoubtedly slower, with from 50 to 75 % of attacked fibres at the home station after 27 days' exposure against 98–100 % at Colombo. The temperature of the water at the latter station during the period of exposure had been 26·5–28 against 14–16° C. at the home station. It is possible, therefore, that the greater rate of attack at Colombo may have been due to the higher temperatures prevailing there, though it cannot be excluded that particularly active cellulose-destroying bacteria may have been present at Colombo. The reason for the rapid destruction of the woo

fabric exposed at Colombo cannot be interpreted on the available evidence since it was found impossible to establish that micro-organisms were the sole cause of decay. It has been mentioned already that Dorée had found that wool fabrics were more resistant to deterioration in salt water than cotton: at Colombo Harbour the reverse was the case.

Summarizing the observations made on fabrics which had been exposed under waterlogged conditions the claim appears justified that micro-organisms are the sole agency responsible for the decay of cellulosic materials exposed to such conditions, at any rate when the relevant organisms are present, either on the exposed fabrics or where they have ready access to the fabrics.

A further conclusion may be drawn from these tests, namely, that fungi, actinomycetes and algae play no conspicuous part in the destruction of cellulose fabrics under waterlogged conditions. Whether the mere saturation of fabrics with water under aerobic conditions, perhaps in combination with diffused light, causes a destruction of fabrics in the absence of direct contact with sources of infection, is a question of some practical interest to the laundering industry, since it is held there that clothing, when it cannot at once be dried, is better protected against damage ("spotting") by being completely immersed in clean water than by being allowed to remain damp.

In theory, Dorée's investigations (1920) would appear to answer the question, since prolonged exposure of fabrics in sea water containing antiseptics failed to cause deterioration. But when no antiseptics are present and when an opportunity therefore exists for a destruction of the fabrics to take place, it would seem possible for infection, carried either by the fabric itself or by soil and dust particles, to initiate decay. However, since fungi appear to have little action on totally submerged fabrics, it is possible that "spotting", which is often held to be caused by "mildew" fungi, may be largely prevented by total submersion of fabrics in water.

(*b*) *Exposure of fabrics to damp, but not waterlogged, conditions*

Both "mildewing" and extensive destruction are known to occur in all types of fabrics which become damp. Burns (1927) states that a moisture content in cotton exceeding 11% is sufficient to initiate deterioration and mentions that where 20% of water is added to raw cotton "heating" and destruction set in. Similar moisture contents would appear to initiate deterioration in flax (Ruschmann, 1923). Wool, on the other hand, does not seem to suffer damage through damp,

beyond "mildewing", until moisture contents of over 24 % have been reached.

Conditions of damp capable of introducing and maintaining 20–30 % of moisture in a fabric are likely to be met with in the soils of most climates and in the shaded humid atmosphere of certain tropical countries.

In exposure trials carried out in Dorset, England, the writers found that cotton fabrics, left in the soil for 5 weeks, had absorbed and retained sufficient water to establish in them a moisture content of over 30 % in spite of the fact that the soil itself never exceeded 15·5 % while the fabrics were in contact with it. Similar and sometimes even more striking figures were obtained in fabrics exposed at other stations. They leave little doubt that in soils of most countries, and presumably in the damp humid atmospheres of some tropical climates, fabrics must be able to absorb moisture to an extent which would be fully sufficient to induce "mildewing" and destruction.

The examination which the writers carried out on cotton, flax, hemp, wool, silk, rayon and jute fabrics, exposed, in the case of the first four, in the atmosphere at Ceylon and Trinidad, and in the soil at Cyprus, Kenya, South Africa and Trinidad; and for the whole range of fabrics at the home station in Dorset, England, confirmed that "mildewing" would set in under the chosen conditions.

The destruction of the fabrics was found to be much more marked in the soil-exposed samples than in those hung in the air at the places of exposure. Columns 6 and 7 of Table I illustrate this.

Table I. *Fabrics exposed at Peradeniya, Ceylon*

Description of sample	Duration of exposure		No. of micro-organisms found per g. of exposed fabric		% of fibres damaged by micro-organisms	
	Shade days	Soil days	Shade	Soil	Shade	Soil
American cotton fabric	365	30	$9{\cdot}3 \times 10^6$	48×10^6	35	88
Egyptian cotton fabric	365	30	$4{\cdot}3 \times 10^6$	$7{\cdot}8 \times 10^6$	40	92
Indian cotton fabric	365	30	$21{\cdot}7 \times 10^6$	$6{\cdot}5 \times 10^6$	46	92
Dew-retted flax fabric	365	58	$12{\cdot}4 \times 10^6$	6×10^6	Approx. 50	Most of fibres damaged
Hemp fabric	365	58	$10{\cdot}4 \times 10^6$	$1{\cdot}1 \times 10^6$	Most of fibres damaged	Most of fibres damaged
Wool fabric*	365	30	$3{\cdot}7 \times 10^6$	$2{\cdot}7 \times 10^6$	No signs of damage	A certain* amount of damage to fibres
Soil sample from place of exposure	—	—	—	$4{\cdot}5 \times 10^6$	—	—

* As mentioned in the first section of this paper, microbiological damage cannot be assessed in the case of wool with the same certainty as in the case of cellulosic fabrics.

Table I shows that the damage to the fibres of the cellulosic fabrics was due to micro-organisms. This claim is based on the result of an analysis of the samples by the swelling test of Fleming & Thaysen (1920) and the Congo red test of Bright (1926).

Little difference was found in the rates of destruction of the various soil-exposed samples between those buried in the soil of a temperate climate such as Dorset, England and those in the tropical climate of, e.g. Malaya. Too far-reaching conclusions should not, however, be drawn from this observation, for the soil exposures at Dorset were carried out during May to August at a time when the soil temperature there did not fall below 15° C. In numerous other trials which the writers have carried out it has been noticeable that the rate of "mildewing" and destruction of a fabric buried in the soil is greatly slowed down during the winter months.

The visible changes suffered by the cellulosic fabrics exposed in the soils and by those hung in the shade were not dissimilar. "Mildewing" was apparent in both cases though decidedly more so in the air-exposed samples than in those from the soil. On the other hand, deterioration with loss of tensile strength was far more serious in the soil-exposed samples than in those hung in the air (see Table I). It is not explained in the table, however, or in any other direct experiment which has been carried out, why soil exposure should be so much more damaging to fabrics than shade exposure, while "mildew" is visually more noticeable on the shade samples than on those from the soil. Though an explanation based on experimental evidence cannot be offered for this, indirect evidence points to differences of moisture conditions in the fabrics as the governing factor.

It has been mentioned that tests had shown that cotton fabrics buried in a soil in Dorset absorbed sufficient water to reach a water content of over 30%, a figure which according to Burns (1927) is more than sufficient to cause "heating" in raw cotton, a process of destruction which leads to a rapid disintegration of the textile properties of the cotton hairs, and which is associated with the growth of cellulose-destroying bacteria (Thaysen & Bunker, 1927). It is questionable whether the bacteria could grow on fabrics with moisture contents below 20–25%. Unpublished experiments which one of the writers (H. J. B.) has carried out indicate that they could not.

Fungi, on the other hand, have been found by the writers (in unpublished experiments) to cause "mildewing" on cellulose pulps containing no more than 10% of moisture. It is not surprising, therefore,

that fabrics exposed in the air at relative humidities of such an order that they are capable of establishing a moisture content of 10 % or more in an exposed fabric should become "mildewed". However, since growth of fungi on a fabric with resultant "mildewing" causes a slow deterioration of the fibres composing the fabric, it is not surprising that even heavy "mildewing" may have failed to deteriorate a fabric to the extent of one exposed to attack by cellulose-destroying bacteria.

After 10 months' exposure in the shade at St Augustine, Trinidad, the test fabrics were weakened considerably more than those exposed at Kuala Lumpur in the Federated Malay States and at Peradeniya, Ceylon, though the actual numbers of micro-organisms found on the Trinidad samples, for instance, were higher than those found on the Ceylon samples.

Here again, differences in humidities at the places of exposure can be correlated with differences in loss of tensile strength, at any rate for the two stations, Trinidad and Malay States, for which data are available. This may be seen from Tables II and III.

Table II. *Relative humidity*

	Federated Malay States		Trinidad	
Av. for month	Morning %	Evening %	Morning %	Evening %
Jan.	87	83	92	66
Feb.	87	87	93	55
Mar.	91	83	96	57
Apr.	84	87	95	54
May	80	88	96	64
June	84	87	95	63
July	84	91	95	72
Aug.	—	96	95	84
Sept.	84	91	96	79
Oct.	83	87	93	77
Nov.	83	—	95	78
Dec.	—	—	94	74

Table III. *Fabrics exposed in the shade*

Type of fabric	Federated Malay States % of damaged fibres	Trinidad % of damaged fibres
American cotton	62	19
Egyptian cotton	85	15
Indian cotton	60	33
Dew-retted flax	59	A small % of total

In connexion with another investigation, one of the writers (H. J. B.) ascertained the moisture content of chemical wood pulp kept for a month at varying relative humidities. The results are shown in Table IV.

Table IV

Approx. relative humidity of atmosphere	Approx. equilibrium moisture content of pulp	
	At room temperature	At 30° C.
%	%	%
98	30	21–22
90	13·5	11
80	10	8·5–9·0
70	8–8·5	7·5–8·0

It is clear that at Trinidad the relative humidity of the atmosphere must frequently have been of an order which caused the exposed fabrics to dry to an extent which reduced the moisture of the fabrics to below the minimum concentration at which fungi can develop. This minimum was estimated by Fleming & Thaysen (1921) as 9%, and by Armstead & Harland (1923) as 7·8%.

The wool samples exposed under the conditions discussed here, i.e. under damp but not waterlogged conditions, were far less affected than those immersed in sea water. A certain harshness was noticeable in the shade-exposed samples, but no tendering of the fabric was noticeable. The wool fabrics exposed in the soil had been tendered and broken up in the manner observed when wool-destroying bacteria act on wool under laboratory conditions.

The silk and rayon fabrics exposed in the soil were all rapidly destroyed, with the exception of the acetate rayon, which remained unchanged during the year of exposure. This high resistance was very noticeable indeed.

The soil-exposed jute fabric suffered rapid disintegration in spite of being partially lignified. The damage caused to the jute could be correlated, as in all the other soil-exposed cellulosic fabrics, with the presence of a large number of micro-organisms, including cellulose-destroying forms.

The types of micro-organisms present consisted chiefly of forms which had no action on cellulose and, therefore, must be regarded as a secondary microflora. Cellulose-decomposing types were present in all the soil-exposed and in some of the shade-exposed fabrics. Their numbers could not be determined quantitatively.

Few actinomycetes were isolated from the shade-exposed samples and these forms obviously did not play an important part in the changes which took place in the fabrics during exposure. Fungi were very much in evidence on the shade-exposed samples, but not in the soil samples. No algae were found. Where "mildewing" or spotting had occurred fungi

could be isolated which, when grown on sterile damp fabrics, could be made to produce such "mildewing" and spotting.

(*c*) *Exposure of fabrics in climates with moderate humidities*

Detailed records are available of the moisture gain of the various fabrics exposed in the shade at Dorset. Reference to Table V shows the

Table V

Station	Total rainfall in in. for the year of exposure	Max. relative humidity in %	Min. relative humidity in %	Av. relative humidity for the year of exposure in %		
				Morning	Noon	Evening
Adelaide	22·54	81	30	53	42·8	64·1
Dorset	28·35	100 (recorded once)	21 (recorded twice)	81·7	—	73·5
Nairobi	26·68	90·7 (recorded once)	36·3 (recorded once)	86·7	55·5	77·8
Pretoria	34·92	84 (recorded once)	20 (recorded once)	77·3	59·0	54·0

moisture conditions in shade-exposed samples which prevailed at the four stations during the year of exposure. In Dorset the moisture found in the three types of exposed cotton fabric never exceeded 20 % and only reached this figure on one occasion, in an Indian cotton fabric. On the days of removal for analysis, all the other samples had moisture contents ranging from 4·7 to 11·2 %. The reason for this great variation is undoubtedly the length of time which elapsed between the last heavy fall of rain and the removal of the sample for analysis. By consulting the relevant data in the records it can be seen, for instance, that the sample of flax fabric which was removed after 9 months' exposure in the shade had been exposed to a fall of rain measuring 19·8 mm. 5 days previously, and that within those 5 days the moisture content of the fabric had been reduced by air-drying from complete saturation to 10·4 %. In the case of another fabric, small falls of rain of the order of a few tenths of a millimetre failed to increase the moisture content beyond 12 %. It is evident, therefore, that except on days of heavy rain the moisture content of the shade-exposed fabrics must have been fairly low, probably of the order of 10–12 %; such concentrations according to Burns (1927) are on the border line of those capable of initiating growth of micro-organisms on textiles.

This was confirmed by the analysis of the various fabrics. In spite of a full year's exposure they contained few micro-organisms, and the damage was restricted to "mildewing" or spotting. Certain types of fungi could be detected in the damaged places. The spotting was more

noticeable on the exposed cotton fabrics than on the heavier linen, hemp and jute fabrics. The viscose rayon fabrics were also extensively spotted, and even the acetate rayon showed occasional spots. In the latter case, however, no fungi could be seen in the spots, which were composed of a dark yellow gum containing large cells, possibly algae. Spotting of a less marked nature than on the cotton fabrics was also met with on the exposed silk and wool fabrics, but this damage can have been of little significance for no fungus mycelium was found in them, and the hairs and fibres of the fabrics were sound.

The bacteriological analyses of the samples revealed much lower numbers of micro-organisms than in fabrics exposed in damper climates. The reduction in numbers was particularly noticeable among the bacteria. It is evident, therefore, that prolonged exposure of fabrics in the shade at places of comparatively low average humidities, between 50 and 90 % R.H., and with normal annual rainfalls of about 30 in., offers little opportunity for micro-organisms to develop, even in warm climates. Where microbiological growth becomes macroscopically noticeable it is restricted to a few species of fungi which cause pigmentation, but no lowering of the tensile strength of the fabric. The spread of these fungi appears to be greater on the cotton and rayon fabrics than on the heavier fabrics of flax, hemp and jute. Wool and silk fabrics are little affected by them.

A certain harshness was observed on the exposed wool fabrics. This could not be associated with microbiological activity but resembled the initial stages of the type of deterioration suffered by this fabric on exposure to sunlight.

(*d*) *Exposure of fabrics in the shade in climates with low relative humidities*

This type of climate was represented by the station at Broken Hill South, N.S.W., Australia. The total rainfall during the year of exposure here was 9·56 in., with a yearly average humidity of the air of 57·6 % at 6 a.m., 40·5 % at noon, and 39·1 % at 6 p.m. Twice during the year the relative humidity rose to 100 %, but it usually deviated little from the yearly average. The minimum for the 6 a.m. reading was 17 %, for the noon reading 9 %, and for the 6 p.m. reading 11 %.

It was surprising to discover in the analyses of the fabrics that the total number of micro-organisms found was of the order of that of fabrics exposed at stations with moderate rainfalls and humidities between 50 and 90 %. Nevertheless, none of the exposed fabrics had

suffered any damage either through loss of tensile strength or through discoloration—"mildewing". The wool fabric had become slightly harsh but a microscopic examination showed that the hairs were normal. Among organisms found, the secondary flora of harmless bacteria and cocci represented the bulk. Actinomycetes were almost invariably absent and fungi were few in number. Cellulose-destroying bacteria were only isolated from one of the exposed fabrics. Algae were not isolated. It may be concluded, therefore, that arid climates offer no facilities for the "mildewing" and destruction of fabrics.

(*e*) *Exposure of fabrics to sunlight in climates with different relative humidities*

The series of exposures included a wide range of climatic conditions from the point of view of rainfall and intensity of sunlight. At Dorset, England, which probably represents the station with a minimum sunshine record, the daily average amounted to 4·6 hr.; at St Augustine, Trinidad, it was 5·6 hr., and at Adelaide 6·5 hr.

The total annual rainfall during the year of exposure is shown in Table VI.

Table VI

Station	Total amount of rain during year of exposure, in in.
Peradeniya (Ceylon)	89·57
St Augustine (Trinidad)	63·1
Pretoria (South Africa)	34·92
Dorset (England)	28·35
Adelaide (Australia)	22·54
Broken Hill South (Australia)	9·56

An examination of the results of the analyses of this series reveals several interesting facts. It appears, for instance, that "mildewing", the degree of microbiological damage suffered by the exposed cellulose fabrics, and the growth of the secondary microflora are all greatly influenced by the annual rainfall, rather than by the amount of sunshine at the place of exposure.

At Trinidad, for instance, where there was a daily average of 5·6 hr. of sunshine, there was very considerable microbiological activity on the fabrics, while at Dorset, England, with a daily average of 4·6 hr. of sunshine, the samples showed far less microbiological damage and lower numbers of micro-organisms than at Trinidad.

On the other hand, it must be admitted that sunlight exercised a considerable action on the microflora of the various fabrics. This is well seen

in a comparison of the shade- and the sun-exposed samples from Ceylon and Trinidad. The shade-exposed fabrics at both stations not only gave higher total numbers of micro-organisms, but a greater percentage of damaged fibres than the corresponding sun-exposed samples. "Mildewing" or spotting was more noticeable in the shade-exposed samples from all stations than in the corresponding sun-exposed fabrics.

In addition to this retardation of damage by sunlight, other effects were observed on the various fabrics. For instance, sun exposure rendered the wool, rayon and silk samples harsh to the feel. The tensile strength of the various cotton fabrics was reduced by the action of sunlight, and even the heavier flax and jute fabrics showed signs of loss of tensile strength after exposure to sunlight. In no case did this action of the sun, which presumably resulted in a degradation of the chemical constituents of the fibres, lead to an increased activity of micro-organisms.

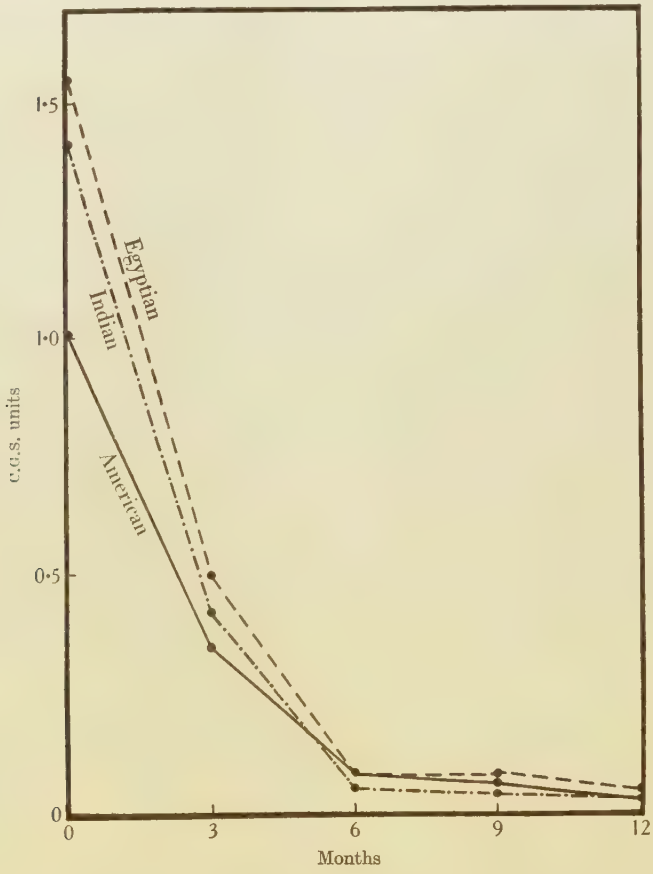
Another general conclusion may be drawn from the analysis of the sun-exposed fabrics. Where microbiological damage, as determined by the swelling test of Fleming & Thaysen, was caused to the fabrics, it was invariably the result of the activity of fungi. In every case which was examined a discoloration—"mildewing"—of a fabric could be ascribed to fungus growth and it was at places of discoloration that a destruction of the fibres could be detected. This observation is corroborated by the almost total absence in the sun-exposed fabrics of cellulose-destroying bacteria and actinomycetes, and by the presence of an unusually small number of bacteria of what has been described as the secondary microflora.

5. The effect of exposure on the viscosity and the reaction of fibres and fabrics

It has already been mentioned that cellulosic and woollen fabrics, when exposed to the action of sunlight in certain tropical countries, suffered a loss of tensile strength due to a weakening of the fibres composing the fabrics. The damage could be shown by appropriate technique (Fleming & Thaysen, 1920) to be similar to the tendering suffered by fibres which had been exposed to the action of chemical agencies such as dilute inorganic acids.

When certain fabrics which the writers had exposed in Dorset, England, were examined, special attention was paid to the action of sunlight for the purpose of determining whether some effect, other than loss in tensile strength, could be detected. It was decided to determine,

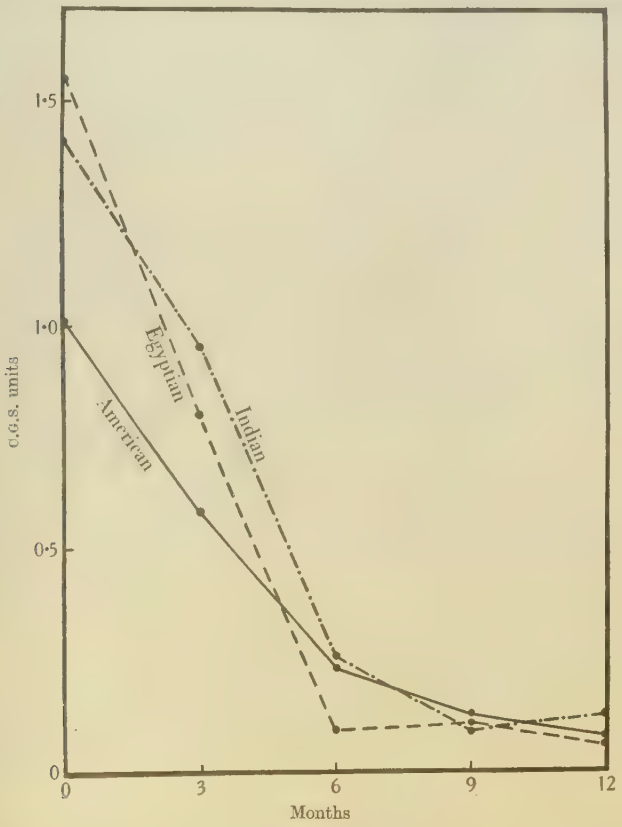
on the one hand, the changes, if any, which might have occurred in the viscosity of the fabrics on exposure, and on the other, the changes in the reaction of the samples. It was argued that changes in viscosity



Text-fig. 1. Viscosity of cotton fabrics after exposure to the sun in Dorset, England. Viscosity measured by Clibben's method in c.g.s. units on a 1% solution. Viscosity of unsterilized samples of fabric: American cotton, 2·79 units; Egyptian cotton, 2·25 units; Indian cotton, 3·90 units.

without any alteration in the reaction of the fabrics might indicate an early stage in the breakdown of the cellulosic substance of the fibres, while changes in both viscosity and reaction would indicate a more advanced breakdown.

The work on the determination of the viscosity of the various cellulose fabrics exposed in Dorset, England, was carried out in collaboration with Dr Barr of the National Physical Laboratory, Teddington, and



Text-fig. 2. Viscosity of cotton fabrics after exposure in the shade in Dorset, England. Viscosity measured by Clibben's method in c.g.s. units on a 1% solution. Viscosity of unsterilized samples of fabric: American cotton, 2·79 units; Egyptian cotton, 2·25 units; Indian cotton, 3·90 units.

with Dr Gibson of the Linen Research Association, Belfast. Dr Barr kindly undertook to determine the viscosity of the cotton samples, and Dr Gibson of the flax and hemp samples.

The results of the viscosity determinations of three types of cotton

fabrics exposed in the sun, in the shade and in the soil are shown in Text-figs. 1–3.

In interpreting these figures it should be emphasized that the careful process of dry sterilization to which the fabric samples had been subjected before exposure (160° C. for 2 hr.) had, in the case of cottons, very materially reduced their viscosity. This reduction became even more marked during the subsequent sun and shade exposure, in the sun more rapidly than in the shade.

Text-fig. 3. Viscosity of cotton fabrics after exposure in soil in Dorset, England. Viscosity measured by Clibben's method in c.g.s. units on a 1% solution. Viscosity of unsterilized sample of fabric: American cotton, 2·79 units; Egyptian cotton, 2·25 units; Indian cotton, 3·90 units.

The soil-exposed samples behaved differently. Here there was an initial steep increase in viscosity which may perhaps be regarded as a recovery towards normal from the loss sustained during sterilization. The rise was followed by a slight decrease which is, perhaps, most representatively shown in the case of the Indian cotton. The final viscosity figures for the soil samples were in all cases higher than those of the original sterilized samples at a time when the samples had to be removed from the soil because of their extensive destruction by micro-organisms. This observation appears to imply that the destruction of cellulosic

fabrics by micro-organisms has comparatively little effect on the viscosity of the materials, an interpretation which is confirmed by the results of the viscosity changes in the exposed flax and hemp samples. Here sterilization had caused no lowering of the viscosity, and a subsequent drop was noticeable only in the sun- and shade-exposed samples, in the former more so than in the latter. The soil-exposed flax and hemp fabrics retained their original viscosity to the end of the trial at a time when the samples were extensively damaged by micro-organisms. These observations support the findings of Searle (1929), who states that flax fibres destroyed by micro-organisms have a viscosity of the order of that of normal fibres.

Two methods were adopted for ascertaining the changes in acidity of the cellulosic fabrics. The first gave a very rough indication of the *p*H value of the samples. It consisted in rubbing a drop of a suitable indicator into the fabric with the help of a glass rod, and observing the change in colour which occurred. The colour change was matched against a suitable standard set. The details of this method were communicated to the writers by members of the staff of the Shirley Institute, Didsbury, Manchester.

The second and more quantitative method consisted in extracting at room temperature 1 g. of a representative sample of the fabric undergoing test with 10 ml. of distilled water of *p*H 7·2. The extraction was continued for 10 min. and the liquid then drained off. The determination of the hydrogen-ion concentration was carried out on the extracts and was measured by suitable indicators. Any change in the *p*H values of the extracts was assumed to be due entirely to changes in the reaction of the fabric from which the extract had been prepared.

It was established that the preliminary sterilization which the fabrics had been given prior to their climatic exposure had caused no measurable change in their reaction, though, as mentioned above, sterilization lowered the viscosity of the cotton fabrics used.

Without giving details of the results, it may be claimed that all the sun- and shade-exposed samples, including even silk and wool samples, showed an increase in acidity during the first 3 months of exposure. This increase was sufficient to lower the *p*H values of the extracts from the original *p*H 6·0 in the case of cotton and 5·0 in the case of jute, to 5·0 and 3·8 respectively in the sun-exposed samples, and to 5·0 and 4·5 in the shade-exposed samples. Subsequent exposure gave rise to little further change, the figures for 3 months being maintained throughout the year of trial.

Soil exposure caused practically no change in the *p*H values of the extracts, indicating that the extensive microbiological damage suffered by the cellulose of the fabrics failed to yield acidic products in measurable quantities or sufficient to prevail over the neutralizing action of the soil itself.

It is not possible to give an explanation for the cause of the increase in acidity of the sun- and the shade-exposed samples, but the fact that this increase had almost reached its maximum within 3 months of exposure at a time when very little damage had been caused by light must imply that light exposure at relative humidities such as those prevailing at the station where the trial was carried out fails to cause a breakdown of cellulose to such an extent that acidic decomposition products result.

6. On certain factors which influence the resistance to decay of fabrics exposed to microbiological deterioration

When the moisture content of a test fabric, as well as the moisture of its surroundings, was kept above 9%, and when the prevailing temperature and other relevant factors such as the place of exposure were maintained constant, it was possible to anticipate that two samples of a given fabric or even samples of closely related fabrics would become affected by micro-organisms at a similar rate and to a similar extent. Cases were not lacking, however, where this did not appear to hold good. The exploration of such cases has led to conclusions which are of a certain practical significance, and merit record.

In the course of an examination of cellulosic fabrics which had been exposed in the soil at Dorset, England, it was found that a viscose rayon fabric of a light voile texture had rotted completely after 3 weeks' exposure, while a heavier and more closely woven material of the same fibre lasted for 4 weeks in the same soil and at the same season of exposure. This and similar observations on other fabrics was interpreted as indicating that the rate of deterioration in soil is inversely proportional to the weight of a fabric, the heavier the fabric the longer would the greater part of its tensile strength be maintained.

Other experiments indicated that additional factors might influence the rate of destruction of fabrics even when moisture conditions, temperature and microflora remained constant and favourable. It was found, for instance, that a sample of loosely woven cotton fabric exposed in the soil at Dorset had lost practically the whole of its tensile strength

after 40 days, while a sample of much more closely woven cotton duck of approximately double the weight lasted for 126 days, that is, more than three times as long as the loosely woven material. The apparent explanation for this, that the outside layers of cotton hairs in the closely woven material prevented moisture from penetrating to the interior of the fabric, could not on further investigation be accepted as satisfactory. For it was found that where a piece of cotton fabric 20×15 cm. was protected on both sides by a shield of glass to prevent moisture penetrating into the fabric, the water content of the material exposed in the ground for 28 days rose to 62 %, while an unshielded control sample of the same material showed only 46 % of moisture when kept under identical conditions. And yet, in spite of the greater water content, the glass-shielded material had retained most of its tensile strength, while the sample of the same cotton fabric exposed in the ground to direct contact with the soil had disintegrated.

This observation suggested further experiments on the effect of shielding of exposed fabrics. Such experiments confirmed that shielding of a fabric by rubber, by a metal such as aluminium, or by glass, made the protected material retain its tensile strength longer on exposure in soil or in the sea than unshielded material of an identical description.

The degree of protection afforded by shielding was determined in a set of soil exposures with a light cotton cloth. This was left partly unshielded in the control sample, or shielded by aluminium, glass, rubber, or vaseline. The various samples were buried vertically in an active garden soil under identical conditions. The temperature of the soil throughout the exposures varied between 70 and 10° C.

The result of this trial is shown in Table VII.

Table VII

Nature of shielding applied to the fabric	No. of days' exposure in the test soil required for the fabric to deteriorate sufficiently to be torn readily by hand
No shielding	28
Shielded with glass	119
,, aluminium	105
,, rubber	105
,, vaseline	32

The moisture content of the shielded fabrics (glass and aluminium) after 28 days' exposure was 15·8 and 16·8 % respectively. After 90 days it was 49 and 44 % respectively. Thus, the shielding of a fabric by inert substances can be shown to retard the normal rate of deterioration of

a fabric by micro-organisms. A similar effect, it may well be assumed, could have been responsible for the great resistance to decay which was shown by the closely woven cotton duck referred to above. The outer layer of hairs or even of threads in this and similar closely woven heavy fabrics may well have acted as a shield protecting the bulk of the interior hairs or threads against decay, and thus reducing the rate of deterioration of the fabric taken as a whole.

Some consideration has been given to the problem of the mechanism by which inert substances are capable of protecting exposed fabrics. It is evident from what has been said that the protection given by shielding cannot be due to an elimination of moisture. Most other conditions which govern the destruction of fabrics by micro-organisms can also be eliminated as a cause, with the exception of the direct contact between an active source of infection and the shielded fabric.

Most workers who have studied the subject of cellulose decomposition have been able to demonstrate that intimate contact between cellulose-digesting micro-organisms and the material which they are expected to attack is essential. It has been suggested (see Thaysen & Bunker, 1927) that the need for this intimate contact is due to the failure of the enzymes produced by cellulose-destroying micro-organisms to function at a distance away from the living cells producing them. Moreover, for a rapid contamination of fresh cellulosic material and for its quick destruction it is essential that it should be in close contact with a large number of active cellulose-destroying micro-organisms. Such large numbers of active cells are, in practice, limited to decaying cellulose debris, the surface of which is covered with masses of cellulose-destroying organisms which adhere firmly to their substrate and thus overcome the difficulty of the limited range of action of their enzymes. When such direct contact is prevented by the introduction of an inert substance between the contaminated cellulose debris of the soil and the fabric to be attacked, infection can occur only through free cellulose-decomposing organisms present in soil waters which may seep into the fabric with the moisture. Since comparatively few such cells are found in soil water the infection of a shielded fabric will progress slowly, particularly as a large amount of growth has to establish itself on fibres before a noticeable loss of tensile strength can be recorded.

That intimate contact between soil and an exposed fabric is established before rotting sets in can easily be observed. An unprotected fabric placed in or on the soil will be found within the first 2 weeks to have a great many particles of soil adhering tenaciously to it. An

effectively protected fabric, on the other hand, can be removed from its place of exposure without adhering soil particles even after many weeks' exposure. Some soil may have become mechanically entangled, but this can easily be removed by gentle shaking.

From time to time preparations have been offered commercially as protections against the rotting of fabrics, the sole function of which has clearly been one of shielding. As typical examples may be mentioned rubber latex and other types of pure rubber compositions, waxes, linseed oil and aluminium soaps. In no case have the writers found such preparations to afford more than temporary protection to a fabric. Their useful application, therefore, must necessarily be limited.

A different type of shielding is met with in jute fibres which are surrounded by parenchymatous and epidermal tissue, sufficiently coherent and continuous to retard the action of such drastic swelling agents as strong alkalies. Hemp may sometimes show such tissues around the fibre bundles, while flax, where badly scutched, can occasionally be seen to have fragments of parenchymatous tissue adhering to the fibres. Cotton hairs are not shielded in any way.

The various exposure trials which the writers carried out with fabrics at Dorset, England, offered an opportunity to determine the protection afforded by the parenchymatous tissue of jute. The results obtained are tabulated in Table VIII, which includes also data on the rate of deterioration of one hemp, two flax and a cotton fabric exposed with the jute fabric under identical conditions in an active garden soil.

Table VIII

Type of fabric	Wt. of fabric in g. per 100 sq. cm.	Actual no. of days taken for the fabric to deteriorate to an extent which allowed it to be torn readily by hand	Calculated no. of days taken for the standard degree of deterioration to be reached when assuming that deterioration would be proportional to weight of fabric and expressing this weight as the strength of the dew-retted flax sample
Dew-retted flax	4·42	20	20
Water-retted flax	4·42	20	20
Hemp	4·42	21	20
Jute	7·47	35	21
Cotton	1·78	14	35

It will be seen that flax, hemp and jute rotted at an almost identical rate while cotton deteriorated somewhat more slowly. The latter conclusion is probably not very significant since the result was obtained on a much lighter fabric than those of the other fibres. Nevertheless, the

results obtained demonstrate sufficiently clearly that the parenchymatous sheath of jute fibres fails to function as a protection against micro-biological attack. Incidentally the results fail to support the view sometimes expressed that flax and hemp fabrics are more resistant to rotting than cotton and that hemp "is a very durable fibre which is not rotted by water" (Matthews, 1924). Nor do the results indicate that the lignin of jute (Cross & Bevan, 1908) is able to delay the rotting of these fibres beyond the time taken for fibres of pure cellulose.

7. On the methods available for the protection of fabrics exposed to climatic conditions

A perusal of the relevant literature gives the reader the impression that the two quite distinct problems of the "mildewing" of fabrics on climatic exposure and of the corresponding damage by fungus attack during manufacture, storage and transit, can be treated as one. Both admittedly are essentially of a microbiological nature, but whilst the former deals with a host of micro-organisms comprising bacteria, fungi and actinomycetes, the latter is restricted to the kinds of lower fungi (Bright, 1925) which may appear on fabrics in the textile mills during manufacture, storage and subsequent transit; Galloway (1930) has given a list of fungi isolated by him from cotton goods. Experience has shown that in many cases the lower fungi can be checked by incorporating a suitable fungicide into a fabric, an expedient which would often fail to influence the progress of damage during climatic exposure, since active fungicides are frequently poor bactericides. There is the further difference between the two problems that climatic conditions will expose a fabric to agencies which, while unimportant during manufacture and transit, may seriously influence resistance to attack by micro-organisms. Exposure to water for instance will leach out of the fabric and eliminate water soluble substances with fungicidal or bactericidal properties. Exposure to heat and wind will aid evaporation of volatile inhibitory substances, and the action of actinic rays may induce changes in these substances which render them less active. Apart from the increased complexity due to exposure to a much greater variety of micro-organisms, climatic exposures, therefore, are much more exacting than exposure to contamination in the mill, and should be dealt with as a separate problem.

Very few instances are known to the writers of the prevention of microbiological attack on fabrics having been attempted by methods other than the incorporation into the fabric of toxic substances. The difficulties already outlined would require such substances to be insoluble,

or at any rate very little soluble, non-volatile and unaffected by the action of actinic rays. A further requirement would be the possession of both bactericidal and fungicidal properties.

The need for the use of only slightly soluble protectives implies that the substances used will function only in the closest proximity to the place of deposition. It is necessary, therefore, that it should be evenly distributed, not only on the surface of the fabric but on every individual fibre and part of fibre composing the treated fabric. In none of the commercial preparations which have been examined have the writers found this requirement fulfilled.

The uneven distribution of the protective agents on a treated fabric is well illustrated in Pl. XLII, figs. 1–4. The specimens were taken from samples of cotton duck which had been treated (1) by the cuprammonium process, (2) by incorporation of copper oleate, (3) by the iron-chromium treatment, and (4) with rubber latex.

In the case of the material treated by the iron-chromium process it was necessary to photograph the fibres at a comparatively high magnification in order to bring out the uneven distribution of the active substance of deposited iron-chromium on the fibres themselves. Text-fig. 3 shows that the bulk of the fibres have perfectly clean and obviously unprotected surfaces.

The protection afforded by rubber is due to "shielding", a mode of protection which was referred to in some detail in § 6. How ineffective the shielding has been in this particular case can be seen in Text-fig. 4, which shows large parts of each thread of the fabric completely free from the black dyed rubber.

In the cuprammonium, and notably in the copper oleate, treated samples of cotton duck penetration has been better, though unprotected parts of the yarn can still be detected. This, no doubt, is due to one thread being superimposed on another, thus preventing the protective substance from reaching the lower thread.

Before an attempt can be made to assess the degree of protection which is afforded by the incorporation into a fabric of toxic substances it is necessary to devise a technique for doing so. Such technique should give the highest degree of accuracy in evaluation, be reproducible, and be completed in the shortest possible time. A further requirement, which in the writers' view is important, is that it should approach as closely as possible the most exacting conditions to which a fabric is likely to be submitted, including leaching, evaporation, aeration, and the interaction of the complex microflora met with under such conditions.

In practice a very great number of tests for determining the resistance of protective substances have been carried out under natural climatic conditions. In this way fishing net and tentage preservations have usually been evaluated. With all its advantages, however, such a method suffers from the serious drawback that the direct results obtained cannot readily be compared since they are often materially influenced by the temperature, and on occasions also by the humidity prevailing at the site of exposure. Thus fabrics exposed in a tropical soil might be destroyed more rapidly than the same fabrics exposed in a temperate climate. Even results obtained at one station in a temperate climate might be found to vary with variations in the seasons of the year. The writers' experience is that this difficulty can largely be overcome by expressing the results, not in terms of actual number of days, weeks or months during which an exposed fabric resisted destruction, but in terms of the ratio between the times taken by a treated and an untreated sample of the same fabric, exposed under identical climatic conditions, to reach the same degree of deterioration. This degree may be chosen arbitrarily, but should be of an order which excludes any doubts as to its nature, whether microbiological or not.

Some workers (Thom *et al.* 1934) have used less comprehensive tests, but these can be justified only when the results obtained can be correlated with the results of exposure to actual climatic conditions.

Searle (1929), like Thom and his collaborators, stresses the importance of the time factor in exposure tests, and for this reason rejects the method of natural exposure in favour of one which can be completed in a shorter time. His technique retains the principle of exposure to the action of soil. Standard-sized strips of the fabric to be tested are brought into close contact with a thin layer of a fine suspension of soil particles distributed on the surface of a Berkefeld filter. The test fabric samples, after being wrapped round the infected moist filter, are incubated under humid conditions at constant temperatures and their tensile strengths determined on a Goodbrand machine at intervals of from 1 to 3 weeks or more. The method, according to Searle, gives consistent results in a shorter time than most natural exposure tests. In the writers' opinion it does not fulfil all the requirements of a reliable test and should not be used, without further exploration, to predict the behaviour of a rot-proofed fabric under climatic conditions. Still more arbitrary are the results obtainable by the methods proposed by Levine & Veitch (1920) and by Thom *et al.* (1934). Both are based on the use of fungi as the only agency of destruction during exposure, in Levine's test of a mixture of

fungi, in Thom's test of one single type. However standardized it is possible to make such methods as regards humidity, temperature and time, they fail to take into account the many other factors which influence deterioration under climatic conditions, and must remain arbitrary until the relationship between them and actual climatic exposure has been established.

The impression which the writers have gained from their work in this field is that too much emphasis has been laid on the importance of the time factor in the elaboration of tests for the evaluation of protective substances. There is much evidence in support of the statement in § 4 (*b*) that but slight differences can be observed in the rate of destruction of soil-exposed samples of one cellulosic fabric whether they be buried in the soil of a temperate or a tropical climate, provided that the soil temperature does not fall below 15° C. and that the soil possesses an active cellulose-destroying microflora. Under such conditions extensive rotting of untreated cellulosic fabrics will take place in 4–5 weeks, a period which is not very much longer than the time required in Searle's tests. Where exposure is carried out in a temperate climate during the winter months, using heavy fabrics for the purpose, the rotting process will naturally be slower.

Further work on the question of standardizing the conditions for a satisfactory technique for the evaluation of rot-proof substances will need to take account of these observations. The difficulties caused by one of them, the weight of the fabric, can easily be overcome by standardizing the type of material used in the tests.

To the writers' suggestion that tests for the evaluation of protective substances should be carried out by exposure of the proofed fabric in an active soil, the objection has been raised that such procedure is unnecessarily drastic; in fact more drastic than conditions likely to be met with in practice. That this is not so has been indicated in the preceding pages, but it may perhaps be pointed out that though a fabric may not throughout its active life be exposed continuously to such drastic conditions, occasions may well arise when it will be; for instance, sand bags and ground-sheets in direct contact with the soil, and fishing nets when left uncleaned on board a trawler after fishing operations have ceased. If a protective substance cannot withstand such conditions it cannot truly be claimed to be able to prevent microbiological destruction.

A considerable number of substances which from time to time have been recommended as protective have been evaluated by the writers by exposure to climatic conditions. Some of the results which have been

obtained in this way have been compiled in Table IX. Where not otherwise stated the test fabric used was a non-sized cotton material weighing 29·7 g./sq. ft. Control and treated material were suspended on aluminium frames measuring 12 × 12 in. and placed vertically either in the top foot of a good garden loam, or for sea exposures submerged below low tide in the sea near the shore.

The value of the several protective substances shown in Table IX is indicated in column 6. Here is recorded the ratio of the times taken for the control and the treated test fabric to deteriorate to the extent that it was possible to tear the fabric between two fingers of each hand immediately on its removal from the place of exposure. This may not be the most desirable method of determining the progress of the rotting of a fabric on exposure, and it is appreciated that in a finally standardized exposure test it may be desirable to replace this by a record of breaking load test on a Goodbrand machine. Nevertheless, the method adopted gave extraordinarily consistent results as may be seen from the data compiled in Table IX.

Table IX

Method of treatment	Soil exposure in days		Sea exposure in weeks		Ratio of destruction between control and treated fabric
	Untreated fabric	Treated fabric	Untreated fabric	Treated fabric	
Latex	—	—	17	35	1 : 2·1
Light yellow aromatic oil	—	—	10	14	1 : 1·4
Aluminium soap	49	49	—	—	1 : 1
Cutch treatment	—	—	10	13	1 : 1·3
Proprietary compound of cutch and chromium	66	158	—	—	1 : 2·4
Sodium silicofluoride	26	26	—	—	1 : 1
Xanthoxin	66	60	—	—	1 : 0·9
Proflavine	25	60	—	—	1 : 2·4
Acriflavine	25	60	—	—	1 : 2·4
2% diphenylamine fluorosilicate	26	40	—	—	1 : 1·5
Iron-chromium treatment of linen fabric	66	127	—	—	1 : 1·9
Iron-chromium treatment of cotton duck	127	235	—	—	1 : 1·9
Copper naphthenate	66	143	—	—	1 : 2·2
Copper oleate	—	—	25	71	1 : 2·8
Acetylation	45	>2645	—	—	1 : >58·8

Of the various materials chosen as protective substances the first three functioned probably through "shielding", the remainder in most cases through toxic action. The various treatments were tested either through sea exposure or soil exposure.

In only one case, that of acetylation, did the adopted treatment

increase the life of the test fabric by more than three times, a rather interesting observation in view of the extensive claims made for some of them. Acetylation was carried out by the method elaborated by Thaysen (1932, 1933, 1936). The results obtained by this treatment confirm those recorded by Dorée (1920) for cellulose triacetate fabrics, and indicate that cellulose acetates even of the order of the monoester or less, which have not suffered visible textile changes and retain their normal strength practically unimpaired, possess unrivalled resistance to destruction by micro-organisms.

That this resistance has been acquired by all the individual fibres or hairs of the treated cellulosic material and by every particle of each fibre is indicated by an experiment carried out on a closely woven cotton fabric which had been mechanically coated with a thin film of cellulose acetate. This fabric when buried under standard conditions in a soil was found to have rotted after 12 weeks' exposure. Examination showed that the active soil organisms had penetrated into the fabric from its ends, which had purposely been left unprotected. From there they had spread underneath the coating of the cellulose acetate layer which, though appearing macroscopically to cover the fabric with a continuous film, had been unable to envelop every thread and, even less so, every hair in the threads, both on its surface and throughout its lumen. It was ascertained that the coating of cellulose acetate on the fabric had not suffered through the exposure.

8. Summary

Various textile fabrics were exposed to a wide range of climatic conditions.

Those exposed to waterlogged conditions were destroyed rapidly. In the case of cellulose fabrics this destruction was due solely to bacteria, and not to fungi or actinomycetes. In the case of wool, the evidence of microbiological action was not conclusive.

Cellulose, wool, silk and cellulose rayon fabrics disintegrated on exposure in microbiologically active soils. The destruction was due to microbiological action, the rate being governed by the moisture content of the soil and, to a lesser extent, by the temperature. Cellulose acetate rayon was completely resistant.

Cellulose and wool fabrics exposed in the shade to very humid tropical conditions were damaged much less rapidly than in soil, although there was extensive "mildewing".

With fabrics exposed for a year in the shade at stations with subtropical or temperate climates, and with a rainfall of about 30 in., the microbiological damage suffered was insufficient to affect the strength of the fabric, but there was much "mildewing". No microbiological damage or "mildewing" was observed in shade-exposed fabrics in arid climates where the annual rainfall did not exceed 9·5 in.

Where fabrics were exposed to the action of sun as well as rain and wind, microbiological damage occurred when the moisture conditions were suitable for the growth of micro-organisms. This damage was invariably less than that of fabrics exposed in the shade at the same station.

Exposure of cellulose fabrics both in sun and shade caused a lowering in viscosity, whereas soil exposure, which causes more extensive microbiological destruction, did not affect the viscosity. All sun- and shade-exposed samples showed a lowering in *p*H value during the first 3 months, but subsequent exposure caused little further change. Soil exposure caused practically no change.

Inert substances such as glass, metals and rubber, when used as a covering for cellulosic fabrics, retard microbiological decay in soil and sea exposures. Some commercial protective substances owe their value to their shielding action. The parenchymatous and epidermal tissues surrounding the fibre bundles of jute exert no shielding effect. In a microbiologically active soil, flax, hemp and jute fabrics decay at approximately the same rate as cotton, which contains no protective tissue.

A provisional technique is described by which the relative merits of "mildew" protective treatments may be evaluated. The only treatment which has been found to give permanent protection in the case of cellulosic fabrics is partial acetylation.

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Fig. 1.

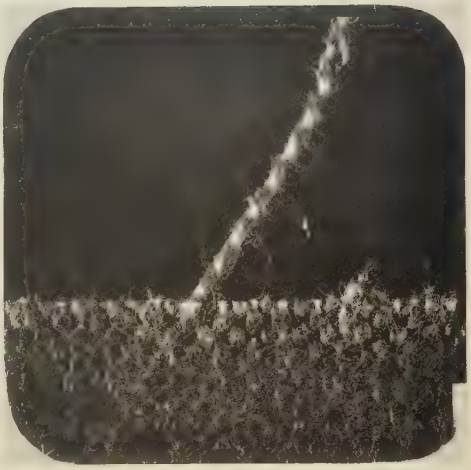

Fig. 2.

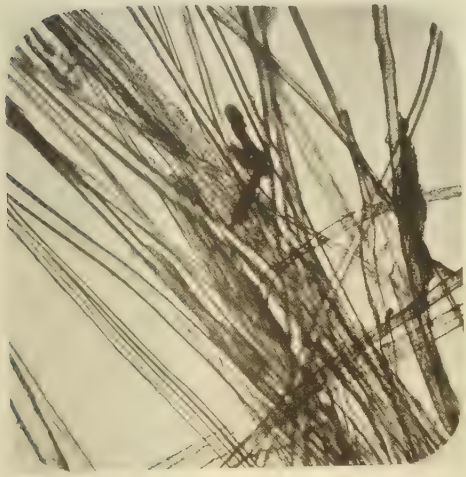

Fig. 3.

Fig. 4.

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EXPLANATION OF PLATE XLII

Uneven distribution of protective agents on treated fabric (cotton duck).

Fig. 1. Treated by the cuprammonium process.
Fig. 2. Treated by the incorporation of copper oleate.
Fig. 3. Treated by the iron-chromium process.
Fig. 4. Treated with rubber latex.

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ON THE LIFE-HISTORY AND ECOLOGY OF *LUMBRICILLUS LINEATUS* MULL. (OLIGOCHAETA)

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(With Plate XLIII and 3 Text-figures)

CONTENTS

Introduction

Lumbricillus lineatus Mull. is abundant and widespread in its distribution, with a varied range of habitats. It is found on the seashore, in streams, polluted rivers and has been recorded from wells. It is also found in the bacteria beds of sewage works where its presence is particularly noteworthy, since recent investigations have shown that it influences the efficient functioning of the beds (Reynoldson, 1939). However, knowledge regarding the life-cycle of this organism is incomplete and its growing importance in applied biology and the ecological aspect of its extremely successful colonization of such a recent and unique environment as the bacteria bed make further information desirable.

The life-cycle has been studied throughout and the reactions of each phase examined experimentally under varying conditions of temperature to ascertain optimum conditions and limiting factors, and the results have been compared with observations in the field. Some study has also been made of the relationship of the worms to other animals in the beds. By this means the life-cycle of the worm has been recorded and evidence obtained regarding the conditions in the bacteria bed which have led to excessive multiplication of this worm.

Habitats

General account

The Enchytraeidae are found in temperate and cold regions. Some groups live in the soil ("xerophilous", Stephenson, 1930*a*), others are typically hydrophilous types, whilst *L. lineatus* belongs to an important group most conveniently termed amphibious. It has the widest range of habitats recorded for any Enchytraeid and is the worm most frequently found inhabiting bacteria beds. It has been reported from these in different parts of England (Friend, 1916), from Belfast (Southern, 1909) and Chicago (Welch, 1914). Specimens in Chicago were named *L. rutilus* by Welch, but his description fits *L. lineatus*. This worm together with species of *Marionina* and *Enchytraeus* occurs in large numbers on the seashore near high-water mark, under stones and in rotting seaweed. They are able to withstand immersion in sea-water and must on occasion have to survive fresh-water. Schmidt (see Stephenson, 1930*b*) found *L. lineatus* in water of varying salinity from 4·5 g./l. upwards and the numbers were not reduced in water containing 39·62 g./l. This remarkable euryhalinity has probably contributed to the adaptability which enables this worm to exist in habitats which few organisms can tolerate. In addition, the severity of the competition for existence is reduced, enabling the worms to be abundant (Stephenson, 1930*c*). Friend (1916) has stated that the Enchytraeids with red blood are most frequently found in polluted localities where the oxygen is likely to be scarce and suggests that the greater respiratory efficiency of haemoglobin might be responsible.

The bacteria beds

A description of the bacteria beds, of the worm and its behaviour has been given (Reynoldson, 1939), so that only the most important points will be recalled. As a habitat for insects the beds have been described by Lloyd (1935) and for worms the following are the most important features: (1) the much reduced fluctuations in temperature of the beds compared with those of the atmosphere and most natural habitats; (2) the constant saturation of the beds with moisture; (3) the great depth of the habitable zone. In view of the abundance of the worms it is evident that these conditions, especially the temperature, must exercise a significant influence.

Life history

Methods

To study the life-history of the worm, observations were made twice weekly at the bacteria beds of the Leeds Sewage Works, Knostrop, from November 1936 to April 1938, and supplemented by laboratory investigations. For experimental purposes, the worms were kept in tubes, 3×1 in., fitted with corks provided with notches plugged by cotton wool for aeration. The bottom third of the tube was covered with black paper to provide protection against the harmful action of light and to discourage the worms from wandering up the tube where they otherwise often dried up. A pad of cotton wool, kept moist, was placed in the bottom of the tube. Small pieces of the alga *Phormidium* (*Cyanophyceae*), previously scalded, were used as food. Under these conditions, two worms could be maintained for weeks in each tube. By selecting worms with large ova ready for deposition, cocoons were obtained in 2–3 days. These were transferred to smaller tubes $1\frac{1}{2} \times \frac{1}{2}$ in. and kept on moist cotton wool. When hatched the young worms could easily be reared on a diet of the scalded alga.

The intensity of breeding was determined by ascertaining the number of sexually mature worms in collections from the beds, the approximate proportions of young worms present and also the approximate abundance of cocoons on the pebbles. Worms with well-developed clitella were taken as being sexually mature, although Stephenson (1930*d*) has stated that one part of the reproductive apparatus may develop whilst the rest lags. This however was not observed here.

Breeding

The breeding activity is apparently most intense during the colder months since at this time sexually mature worms predominated in the collections; young worms were common and cocoons abundant on the pebbles in the upper 6 in. This time agrees with that for the Enchytraeidae in general. There is no clearly defined season for breeding in *L. lineatus* and Oligochaetes in general as in many other animals, but merely a tendency for it to be more intense at one period of the year, and sexually mature worms are to be found at all seasons at Leeds. The reason for the existence of a seemingly more favourable period is at present undetermined. For some species of *Nais* it has been stated that oxygen production by algae was the effective factor, but this is not constant even for the Naididae (Stolte, see Stephenson, 1930*e*) and determining factors remain obscure.

Copulation was observed and found to agree with Ditlevsen's account (see Stephenson, 1930*f*). The two copulating worms lie head to tail, with the ventral surfaces in apposition and the penes which are the muscular terminal portions of the male ducts are inserted into the spermathecal apertures of the partner. The spermatozoa are introduced and stored in the spermathecae. When the cocoon secreted by the clitellum and containing the ova is carried forward over the spermathecal aperture, sperms are passed into it and the eggs fertilized. An unusual feature of *Lumbricillus* and other genera of the *Enchytraeidae* is the opening of the spermathecae into the oesophagus. It is supposed that any excess of sperm may be passed into the gut for digestion and absorption.

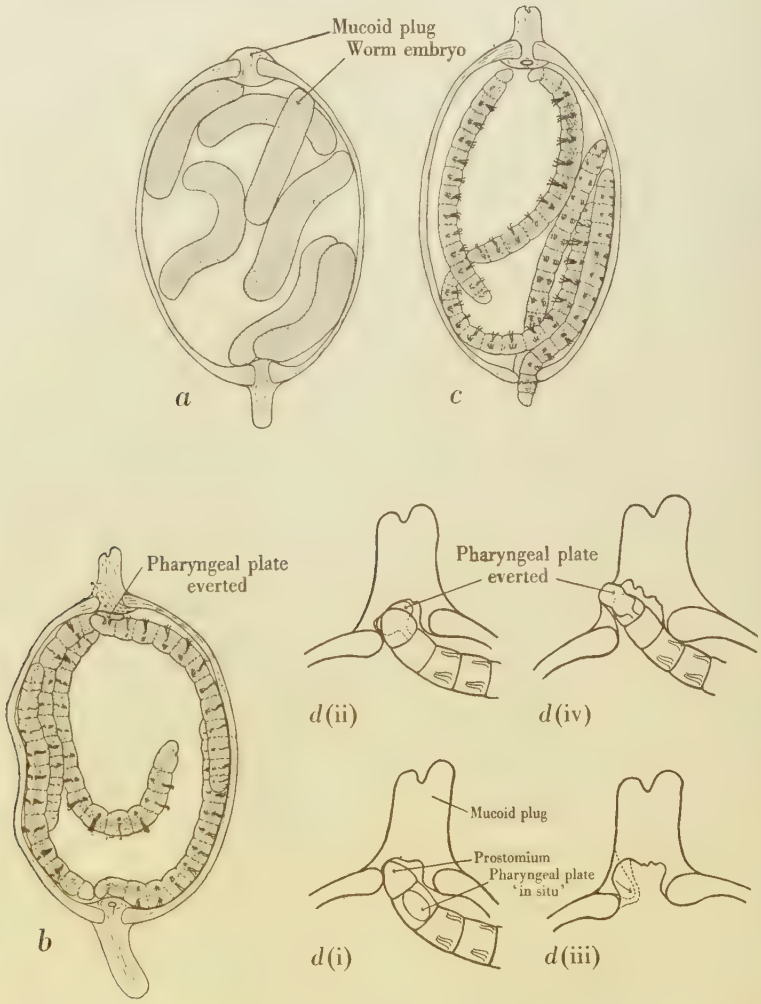
The cocoons

The cocoons are firmly attached to the substratum and this enables them to resist the constant downward trickle of sewage through the beds. They are deposited in the sheets of alga growing on the surface stones and upon the stones below, being most abundant on those with rough faces (Pl. XLIII, fig. 2). In the laboratory, the cocoons are found adhering to the strands of cotton wool, to the sides of the tube and to the pieces of alga.

The cocoons (Text-fig. 1) are ovoid and average $1{\cdot}1 \times 0{\cdot}75$ mm. but considerable variation was found, the length 1·3–0·9 mm. and the breadth 0·9–0·56 mm. By way of contrast the cocoons of *L. maximus* are much larger, being $1{\cdot}75 \times 1{\cdot}4$ mm. Thickenings of the wall occur at each end where closure has been effected by mucilage plugs during deposition. The wall of the cocoon is smooth and transparent and also composed of mucoid material. The ova are distinctly visible inside the cocoons as white, oval or rounded bodies and vary in number from 1 to 20 although 4–9 is the most usual. Ditlevsen records higher figures for this species (30–35). Extreme variation was found in the proportion of fertile to sterile eggs in a cocoon and the greatest number of worms observed to hatch was 14. There is no relation between the number of eggs and the size of the cocoon. The ova are bathed in a colourless fluid unlikely to be albumenoid (Stephenson, 1930*g*).

Development

The rate of development naturally varies with the temperature and at 12–14° C. elongation began one day after deposition. The embryos gradually elongated and by the third day they had become active in the cocoon. The worms are at first opaque and white in colour, due to the



Text-fig. 1. *a*, cocoon 3 days' old at 15° C; *b*, cocoon showing pharyngeal plate everted—direction of boring indicated by arrow; *c*, cocoon showing hatching. Stages in hatching process: *d*(i), boring with prostomium before everting pharyngeal plate; *d*(ii), pharyngeal plate everted; *d*(iii), showing effect of sucking on wall; *d*(iv), showing pharyngeal plate piercing wall.

presence of yolk, but gradually become transparent as it is absorbed. Hatching at this temperature normally takes place 9–11 days after deposition. During development there is no increase in size of the cocoon.

Hatching

It was at first thought that the young worms hatched from the cocoon by lying closely against the wall and exerting an outward pressure. This same idea was also expressed by Roule (1889) who worked on an Enchytraeid which was probably a Lumbricillid. Later, however, by closely examining the cocoons it was observed that there was a definite procedure leading to hatching. This as far as the writer is aware has not previously been described. The preliminary stages consist of "nosing" with the prostomium in the region of the polar plugs, later activities being concentrated on only one of these. After one or two partial evertions the pharyngeal plate (see below) is everted over the region of the plug and the worm sucks vigorously. The suction power is considerable since the outer end of the plug quivers noticeably. The worm then proceeds to bore into the wall, again sucking with the everted pharynx. After two or three efforts by one worm it will move away and its place will be taken by another, so that an almost continuous boring and sucking is kept up. As a result, the worms gradually push and tear their way into the junction between the cocoon wall and the plug (Text-fig. 1 *b*, *d*) and as the wall in this region becomes thinner the effect of each sucking is to pull the wall inwards as shown in the diagram (Text-fig. 1*d* (iii)). Finally, it becomes so thin that as the pharynx is everted it pierces the wall and the worm accomplishing this crawls out to be followed by the others as they discover the rupture. The approximate time from the beginning to the end of this process is 3 hr. It is of interest that the worms attack the weakest point in the cocoon structure. This can easily be shown by placing a cover slip over a cocoon and pressing lightly, when the rupture almost always takes place at the junction of the wall and the plug.

The pharyngeal plate is a projection of the dorsal gut wall into the gut lumen. It consists of tall, columnar cells and is well supplied with muscles. Its structure and association with certain glands has been described by Stephenson (1922) who has also given a number of suggestions as to its function (1930*h*). It is therefore of interest to record this instance which was not mentioned by him. The worms when hatched are about 1 mm. in length comprising 13 segments, with well-developed setae numbering 2 per bundle. They are very active and commence to feed at once.

There are two interesting features about the development. In the first place, in 130 out of 200 cocoons all the eggs did not develop and in some none at all: it is probable that these eggs had not been fertilized. This was noted both in cocoons deposited under artificial and natural conditions. Secondly, during development, the active worms attacked the sterile eggs and fed upon them and these usually disappeared in a day or so, although in some cases remains of sterile eggs were present at hatching. Similar observations have been recorded for *Tubifex tubifex* (Stephenson, 1930*i*). Worms feeding in this way should be larger than those without extra provisions, so measurements were made of hatching worms and correlated with the quantity of food available as sterile eggs. Table I shows the results of the observations; the worms are divided for convenience into three groups, based on the number of sterile eggs available per 10 worms.

Table I. *Size of worms in relation to food available in the form of undeveloped eggs*

	Group 1	Group 2	Group 3
Ova available per 10 worms	0	0–5	5
Av. size of worm, sq. mm.	0·155	0·186	0·243
Length × breadth	±0·0029	±0·00307	±0·010
Range in size	0·084–0·157	0·117–0·259	0·084–0·416
No. of worms measured	62	75	31

These results show an increase in size of the worms correlated with increase of food in the form of sterile ova which is statistically significant. Lack of uniformity in the size of the worms hatching from a single cocoon was great in some cases, as the following examples show:

Cocoon 1. 0·143, 0·248 sq. mm.
Cocoon 2. 0·084, 0·127, 0·166, 0·167 sq. mm.
Cocoon 3. 0·094, 0·148, 0·173, 0·315 sq. mm.

and accounts for the wide range in size of the worms in the above groups (Table I). These observations may indicate the reason for the great variation in length found in the adult worms (9–17 mm.) and might be of importance in the study of their evolution since they seem to show the existence of intra-specific competition and are analogous to the cases of intra-uterine competition cited by Huxley (1936), in this case competition for the sterile ova replacing that for the parental food supply.

Biotic factors—predacity and parasitism

Descriptions of the fauna of these beds have been given in previous papers (Lloyd, 1935, 1937; Reynoldson, 1939) and one of the interesting features is the comparative scarcity of definitive predators, especially those feeding upon the worms. It has been suggested by Lloyd (1937) that in times of food scarcity in the beds some of the dipterous larvae may become carnivorous. This is most likely to occur at off-loading periods and in the summer when the insect population is at its height. In view of the extreme difficulty of making accurate field observations, the possibility has been examined in the laboratory with a series of combinations of the dominant larvae and the worms and cocoons. A full description of the technique and results is given elsewhere (Lloyd *et al.* in the Press), but it might briefly be said that the attacks of the larvae upon the adult worms were insignificant but there is a distinct possibility that they feed readily upon the cocoons in the beds.

Nematodes were suspected of attacking the cocoons but evidence was never obtained. Their eggs have however often been found deposited on the wall of the cocoon.

Parasites of the worms and cocoons

The adult worms generally harbour a ciliate (*Anoplophryinae*) in the fore-gut, an association which seems to be of frequent occurrence (Stephenson, 1925). No pathogenic condition was evident but Stephenson has reported a case in which the alimentary canal was blocked by the ciliate and the wall degenerate. Parasites belonging to the Monocystis group of the Sporozoa invariably occurred in the segments containing the testis lobes.

Empty cocoons collected from the beds were often swarming with ciliates but these were probably attracted by the remains of the ova after hatching and are not parasites. The cocoons afforded an anchorage for Vorticellids and other peritrichous ciliates. During experimental studies of development it was noted that the cocoons were sometimes attacked and the eggs destroyed by a ciliate of the genus *Glaucoma*.

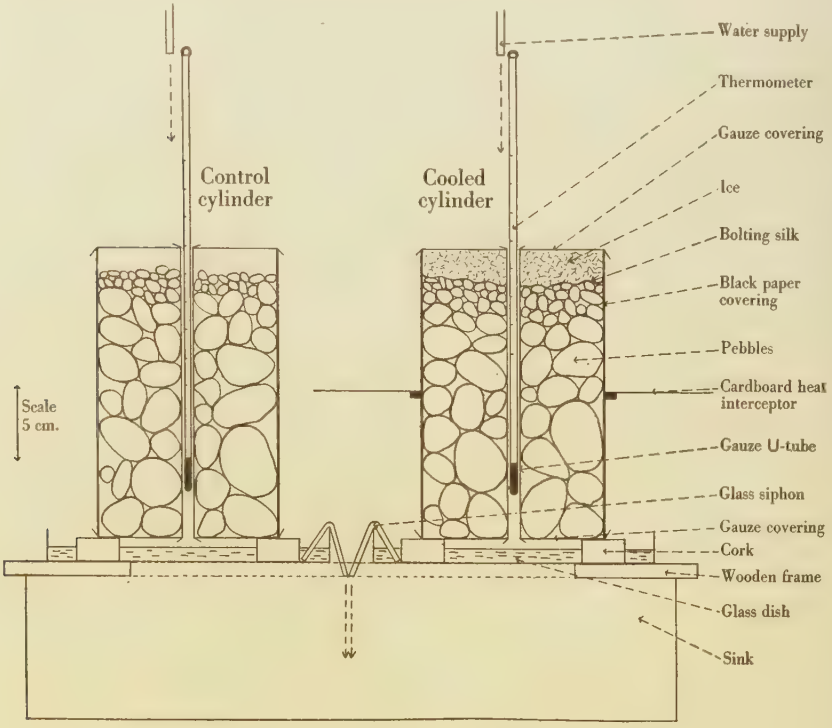
Experimental study of worm behaviour and fecundity in relation to temperature

I. *Temperature and worm behaviour*

Detailed investigations of the worm numbers in the surface alga showed that during periods of exceptionally cold weather the worms migrated from the surface to the warmer depths (Reynoldson, 1939).

This reaction was tested experimentally by using models of bacteria beds in one of which a moderate temperature could be maintained throughout, while in the other a graded temperature could be arranged.

Description of apparatus (Text-fig. 2). A glass cylinder 5 in. diam. and 8 in. deep was filled with clean stones taken from the bacteria beds. Small stones ($\frac{1}{4}$–$\frac{1}{2}$ in.) were used for the upper inch to increase the spread of water arranged to drip on to the surface, but the remainder were 1–3 in. in length. The cylinder rested on perforated



Text-fig. 2. Diagram of the model beds.

zinc, supported by three corks in a shallow circular glass dish. A siphon was arranged to empty water from this dish as it collected, conducting it to a sink. The upper surface of the glass cylinder was covered with perforated zinc to disperse further the water which was supplied as a rapid drip from a height of 6 in. above the gauze. By this means it was possible to keep the stones thoroughly wet during the experiment.

A piece of perforated zinc bent into a U-shape in transverse section, allowing a thermometer to slide up and down inside, was placed in the middle of the cylinder. It was fastened below and kept in place by the pressure of the stones packed around it. Another cylinder was arranged similarly as a control, but a space of 1 in. was left

free above the medium. Both these cylinders rested on a wooden framework over a sink.

A special thermometer, calibrated to enable temperatures to be taken at any desired depth, was used. The U-shape of the thermometer's setting ensured that the temperatures recorded were those of the stones.

In the cylinder in which the graded temperatures were desired, a layer of bolting silk was placed over the upper surface of the stones, and ice, broken into small pieces, tightly packed over it. To keep the stones as wet as in the control cylinder, it was found necessary to pipette ice-cold water over the surface every ½ hr. The cylinders were covered with black paper to prevent any distracting influence due to lateral light.

Preliminary tests showed that 2–3 hr. after the ice had been applied the cooling effect in the lower half of the cylinder was great, whereas it was desired to simulate the conditions in the bed where the surface cools but the depths remain warmer. Therefore two lamps were arranged to throw a suitable heat on to the lower half of this cylinder, the upper half being protected by a platform of cardboard resting on corks glued to the black paper covering of the cylinder. Further tests showed that the apparatus now gave the required temperature grading.

Method. The drip of water was started some time before the beginning of the experiment so that the medium was thoroughly wet. Worms freshly collected from the bacteria bed were placed on the upper surface of the medium of both cylinders, and left to distribute themselves overnight. Next day the control cylinder was left undisturbed but the supply of water was turned off in the other and ice applied to the upper surface. Temperatures were taken every ½ hr., in the case of the control at the top, middle and bottom levels, and in the other at quarterly "depth intervals". The experiment occupied 4–6 hr., and at the end of that time the medium from the top, middle and bottom thirds of each cylinder was removed into separate dishes and the worms collected and counted. The few worms washed out of the beds were counted in with those from the bottom third.

Table II. *Distribution of the worms and the temperatures in the two cylinders*

	Distribution of worms in % of total used				Temp. °C.		
	Top	Middle	Bottom	Worms used	Top	Middle	Bottom
Exp. I. Cooled cylinder	12	54	34	800	1·5–5·5	5·5–8·3	8·3–9·3
Control cylinder	68	23	9	630	11·5	11·5	11·5
Exp. II. Cooled cylinder	39	39	23	950	2·2–6·8	6·8–8·5	8·5–10·5
Control cylinder	43	48	9	940	13·8	13·8	13·8
Exp. III. Cooled cylinder	19	25	54	510	2·8–6·0	6·0–8·0	8·0–8·5
Control cylinder	47	29	26	740	11·0	11·0	11·0
Exp. IV. Cooled cylinder	28	19	54	980	1·3–5·0	5·0–7·8	7·8–9·0
Control cylinder	—	—	—	—	—	—	—
Exp. V. Cooled cylinder	12	31	57	980	1·4–4·3	4·3–6·5	6·5–7·5
Control cylinder	76	12	12	800	10·8	10·8	10·8
Av. Cooled cylinder	22	33	45				
Control cylinder	55	44	13				

The results are given in Table II. In the cylinder of uniform temperature, at the end of each experiment the majority of the worms was gathered in the upper half, but, in the cylinder cooled at the top, in each case the majority of the worms had migrated to the lower, warmer half, except in Exp. II where the top temperature was rather higher. This confirms the belief that the downward migration of the worms in the beds in cold weather is due to a thermotropic response.

II. *Temperature and worm fecundity*

The reduced range in daily and seasonal temperature fluctuations compared with those of the atmosphere and most natural habitats is one of the striking features of the bacteria bed, and since the breeding occurs principally in winter the relationship of temperature to worm fecundity was examined.

The experiments were carried out during the period of most active breeding of the worm in an apparatus devised by Prof. E. A. Spaul for maintaining graded temperatures. This consisted of a long zinc trough divided into compartments, one end being in an ice box and the other in a controlled warm water bath. The trough was enclosed in a case packed with felt and cork and the compartments had tightly fitting cork lids. In spite of this insulation there was a slight temperature swing of 1–2° C., the means about which are recorded in the following account.

Cocoon deposition and egg sterility. Worms were kept in the apparatus at temperatures of 1, 5, 7, 10, 15 and 20° C. approximately for a period of 4 days. The cocoons and contained eggs deposited during this time were counted and the former are expressed in Table III as a percentage of the number of worms used.

Table III. *Showing the effect of temperature on cocoon deposition and egg production*

Av. temp. ° C.	No. worms used	Total no. cocoons observed	Av. no. eggs per cocoon	% cocoons/ worms
1·5	150	0	—	0
4·5	160	17	7·0	11
7·4	149	57	6·0	38
9·8	106	54	6·0	51
15·0	128	86	7·0	67
20·2	158	106	7·0	67

The results show that increase of temperature up to 15° C. causes an increase in the rate of production of cocoons and eggs (since the average number of eggs per cocoon varies slightly), presumably due to greater physiological activity with increasing temperature. The most pronounced effect is caused by a temperature of 5–6° C.

The percentage of sterile eggs deposited at each of these temperatures has also been observed and the results indicate that a temperature of 20° C. causes an increase in sterility (Table IV).

Table IV. *Showing effect of temperature on egg sterility*

Av. temp. °C.	Total eggs observed	% sterile
4·5	560	32
7·4	588	29
9·8	522	24·5
15·0	681	26
20·2	728	42

This is not likely to be of importance to the worm in maintaining itself for two reasons: first, the worm breeds mainly in the winter, and secondly, bed temperatures of 20° C. have not been recorded over a period of 5 years' observation.

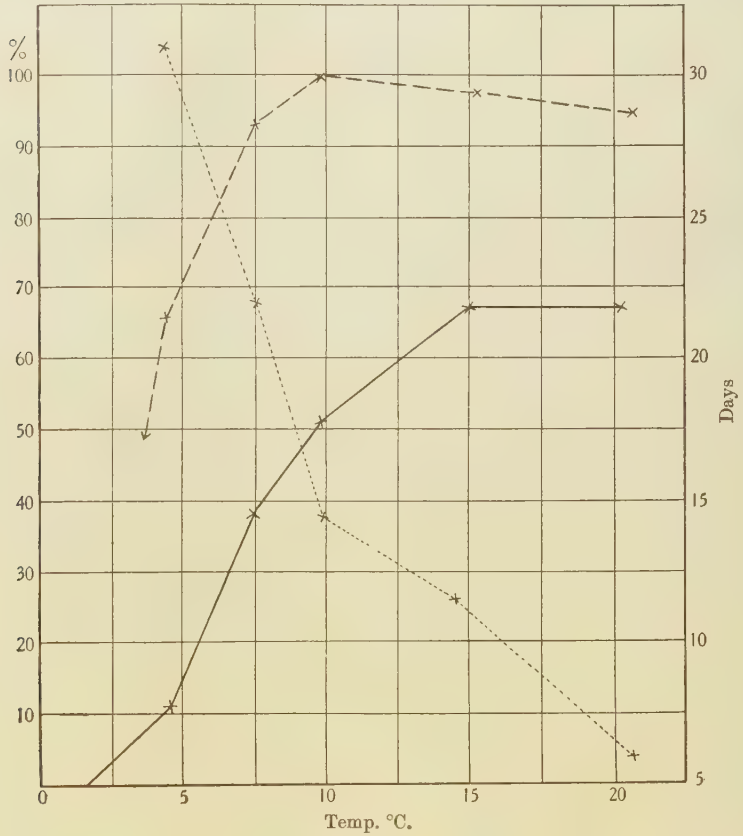
Development and hatching. With increasing temperature the rate of development increases (Text-fig. 3 and Table V), but at temperatures of 1–2° C. only 9 out of 20 cocoons showed eggs developing and of these only one hatched. These cocoons had been deposited at 7° C. owing to the difficulty of obtaining them below this temperature, and the cotton wool on which they were kept had been moistened with sewage. Here again the greatest effect within the range of viability is in the region of 5–6° C.

Two series of approximately 20 cocoons were used at each temperature gradient and it was found that for a number of cocoons the worms within failed to rupture the wall and died. The percentage failing to hatch was fairly constant (26 %) for temperatures of 7° C. and above but at 4° C. it was as high as 80 % (Table V). For these experiments tap-water had been used to moisten the cotton wool on which the cocoons had been kept, but in view of the considerable mortality resulting, boiled bacteria bed effluent was tried instead. With this the number of cocoons from which the worms failed to hatch was reduced to 3 % for temperatures of 7° C. and above, and at 4° C. was as low as 20 %. This indicates that some property of the bed effluent is more favourable to hatching than tap-water and, in addition, the time from deposition to hatching is slightly reduced. The reason for this is being investigated.

Discussion

The bacteria beds of sewage works form an artificial environment to which many species of Oligochaetes, both as free-living forms and in cocoons, must constantly be carried, especially during periods of heavy

rainfall. At Leeds only three species have been able to colonize this environment successfully; *Lumbricus rubellus* Hoff. and *Lumbricillus lineatus* in the beds themselves and a species of *Nais* in the channels below. It is of interest to enquire why *L. lineatus* alone of all the small worms of this



Text-fig. 3. Showing the rate of cocoon deposition development and hatching in relation to temperature, taken from Tables III & V. ——, % cocoon deposition; – – – –, % hatching, sewage; -------, days to hatch, sewage.

class is able to thrive in these beds. Structurally and in its life history this worm shows no specialization which might account for it, but physiologically the worm shows a wide range of adaptability. The known facts of its distribution show that it is adaptable to a wide range of physical and chemical conditions, since it can live on the seashore or at

Table V. *Rate of development and number of cocoons hatching at different temperatures, for sewage and tap-water*

Medium	Temp. °C.	No. of cocoons observed	No. of cocoons hatching	Days to hatch	% hatching
Tap-water	4·3	13	5	41	38
	4·5	15	3	28	20
Sewage	4·4	20	16	33	80
	4·5	7	4	28	57
Tap-water	7·1	17	12	25	71
	7·4	21	16	24	76
Sewage	7·5	24	22	23	92
	7·5	19	19	21	100
Tap-water	10·0	21	13	15	62
	9·9	20	14	19	70
Sewage	9·8	23	23	16	100
	10·1	20	20	13	100
Tap-water	15·9	21	14	11	67
	15·3	22	19	9	86
Sewage	15·5	29	29	10	100
	15·0	23	22	13	96
Tap-water	20·7	24	16	7	67
Sewage	20·6	21	19	6	90
	20·3	28	28	6	100

the margins of streams. Information is needed upon the following three points before an understanding of the relationship between the worms and the beds is possible.

(1) The general resemblance which the beds bear to the normal environment of the worm.

(2) The features of this special environment favourable to the worm's existence and allowing a density of population to be reached rarely equalled in natural habitats.

(3) Factors in the special environment, structural, physical, chemical, or biotic which offer resistance to the worm's multiplication.

(1) The beds are saturated with moisture and the worms are subjected to periodic washing, though at more frequent intervals than would be experienced under natural conditions. They are exposed to fluids of rapidly varying osmotic pressure. They must be able to resist the downward flow of sewage when resting on pebbles often devoid of any growth in which they can burrow, though on the surface where the water flow is most powerful the growth of *Phormidium* (*Cyanophyceae*) provides an anchorage. The force to be resisted is less than the suction of the tide, but support, except at the surface, is less secure. There is an abundance of decaying vegetable matter for food.

(2) The structure of the beds is an important factor contributing to the worm's success. The depth prevents complete drying out when the beds are not in use—actually only the upper 4–5 in. dry out—so that the worms have a retreat against desiccation rarely found in their natural habitats where dry spells must cause a high mortality.

The temperature of the beds is much higher in winter than that of the surrounding atmosphere and most natural habitats, due to the protection afforded by the bed structure itself and the heat of the vital activities taking place within. This gives the worms a retreat from extreme cold and is favourable to a high rate of breeding, since they breed mainly in winter. It has been shown that only below 6° C. is breeding seriously affected and records show that only once has the average monthly temperature of the beds fallen below this critical figure over a period of 5 years. This must occur frequently under natural conditions.

The depth of the habitable zone affords refuge from the birds (mainly starlings and meadow pipits) which feed on the surface of the beds often in large flocks. Bird life is also abundant by streams and on the seashore but the protection is not so adequate.

Further, the bacteria beds are peculiar by reason of the comparative rarity of natural predators in the fauna. Experiments have shown that certain of the dipterous larvae living in the beds may feed upon the cocoons in time of food shortage but, nevertheless, it is reasonable to say that here predators are very few, enabling the worms to increase almost unchecked by such a depleting factor.

(3) Factors offering resistance to the worm's multiplication are relatively few in the bacteria bed. The osmotic variations of the sewage are no greater than those occurring on the seashore and cannot be regarded as an unfavourable factor, but the peculiarities of the chemical composition of the sewage need to be tolerated. The sewage at Leeds is not characterized by any special trade waste nor is it subject to any drastic chemical treatment, and it is, therefore, more suited to the worm which can evidently tolerate a wide range. Worms are much less numerous at Huddersfield where drastic chemical treatment is carried out, and trade wastes from dye works form a large part of the sewage.

Sewage is characterized by a lack of dissolved oxygen and it is a general feature of Invertebrates that those with haemoglobin in the blood more frequently inhabit places where the oxygen tension is low. It has been shown (Leitch, 1916) that *Planorbis* and *Chironomus* larvae (species not stated) utilize the oxygen binding properties of the haemo-

globin to enable them to live at the low oxygen tensions of their habitats. Friend (1916) has suggested that the possession of haemoglobin by some Enchytraeids has enabled them to inhabit polluted areas. Observations in the present case indicate that haemoglobin is unnecessary in the bacteria beds, since although the sewage delivered on to the beds is usually devoid of dissolved oxygen, the worms are only covered by a thin film for most of the time and breathe atmospheric oxygen which is abundantly supplied by the aeration of the beds. The haemoglobin might help worms to pass through the various regions of the sewage plant to the beds where they are immersed.

Reference has been made to the need for resistance by the worms and cocoons to the flow of sewage. The worms avoid being washed down by their activity and the cocoons are firmly attached to the pebbles. This is of interest since it is only in the genus *Lumbricillus* of the Enchytraeids that the cocoons are recorded as being attached to the substratum (Stephenson, 1930*i*).

This account shows clearly the unique type of environment formed by the bacteria bed with regard to the Enchytraeid worms and *L. lineatus* in particular. It is a habitat which encompasses the main features of the wide range of habitats of this worm in nature, especially its amphibious character, the presence of a water flow, the wide range of chemical conditions and an abundance of suitable food. In addition it possesses definitely favourable features to the worm's existence, such as a higher temperature in winter and a scarcity of predators. Not only does it favour the worm in this direct way but also in an indirect manner, for in the complex of factors are those which inhibit colonization by other Enchytraeids (the nature of the habitat naturally inhibits purely aquatic types such as *Nais*), the most efficacious of which is probably the peculiar chemical conditions which few are capable of withstanding. Thus, this worm by virtue of its great tolerance can multiply unchecked by competition with related species in this artificial environment and take full advantage of the favourable conditions which bacteria beds offer.

Summary

1. A survey of the natural and artificial habitats of *Lumbricillus lineatus* Mull. shows a wide range and toleration of diverse chemical conditions.

2. The bacteria beds are described in relation to the needs of the worm.

3. An account is given of the life-history of the worm including breeding season, copulation, cocoon deposition, development and hatching. The latter is given in detail since it is believed to be new. It demonstrates a use of the eversible pharyngeal plate. Experiments show that the hatching worms increase in size with an increase in quantity of food available within the cocoon in the form of undeveloped ova.

4. A short account of the parasites and possible predators is given.

5. Experiment shows that the worms migrate from low temperatures to higher, a reaction observed in the beds during cold weather.

6. Temperatures below 6° C. affect adversely: (*a*) cocoon deposition, (*b*) rate of development, (*c*) hatching. The beds, in contrast to natural habitats, rarely fall below 6° C. Egg sterility is increased at a temperature of 20° C.

7. The bacteria beds form an environment for this worm which includes all the main features of its natural habitats. The higher temperature in winter, the protection from desiccation and birds, and the scarcity of natural predators allow excessive multiplication of the worm. Further, certain factors in the beds, probably the peculiar chemical conditions, prevent colonization by other similar worms so that competition with other Oligochaetes is at a minimum. Thus, by virtue of its great degree of physiological adaptability, this worm is able to thrive in the bacteria beds to an extent not found in any natural habitat.

My thanks are due to Dr Ll. Lloyd for many helpful suggestions in the course of this work and to Prof. E. A. Spaul for his valuable assistance in the preparation of the paper. Mr J. T. Thompson, Manager of The Leeds Corporation Sewage Works, kindly gave permission to visit the bacteria beds, and in addition to recording the bed temperatures has helped in every possible way. I am grateful to Dr W. S. Bullough for the photographs of the worms and cocoons.

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Fig. 1.

Fig. 2.

REYNOLDSON.—On the Life-History and Ecology of *Lumbricillus lineatus* Mull. (Oligochaeta) (pp. 782–799)

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EXPLANATION OF PLATE XLIII

Fig. 1. A characteristic clump of worms on a stone beginning to break up—the white patches visible on some worms indicate the segments containing testes and ovaries. ×2·5 approx.

Fig. 2. Photograph of stone from beds showing cocoons attached. ×3·0 approx.

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STUDY OF *BYSSOCHLAMYS FULVA* AND CONTROL MEASURES IN PROCESSED FRUITS

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(With 2 Text-figures)

CONTENTS

I. Introduction

The principle underlying the preservation of fruit in cans or bottles is to seal the raw fruit immersed in a sugar solution in the container, and sterilize it by heat. Experience has shown that, as a rule, moulds and yeasts can be destroyed by heating the cans for a time which gives a palatable product of attractive appearance, and heat resistant bacteria are rendered innocuous because of their inability to grow in acid media such as fruit juices. Spoilage of preserved fruit by micro-biological activity has been of comparatively rare occurrence and could usually be traced either to mistakes in the routine of heating, or to micro-organisms entering the container after sterilization, owing to unsatisfactory sealing. When such spoilage has occurred it has been accompanied by gas production, leading to "blowing" or bulging of the ends of the container, and so the trouble could be detected by the external appearance of the can.

A form of spoilage caused by a mould with heat resistant ascospores, *Byssochlamys fulva*, Olliver & Smith (1933), has been encountered in recent times and has been described by Hirst & McMaster (1933) and by Olliver & Rendle (1934). In affected cans the texture of the fruit is very soft, and any shaking of the container, such as happens in transport, causes the fruit tissues to break down and become a pulpy mass. Disintegration may even take place while the containers are in the store. As a rule there is not sufficient gas production in the container to blow out the ends, so that affected cans are unrecognizable until they are opened. This is of commercial importance, since such cans may reach the consumer and cause prejudice against the products of the manufacturer concerned and even against canned goods in general.

Information about the physiology of *B. fulva* is given by Olliver & Rendle (1934), who state that it is a saprophyte which is easily cultured on media and grows especially well on fruit juices of acid reaction and of approximately 10 % sugar content. It is unable to attack living fruit tissues but disintegrates those of preserved fruits. The presence of the fungus in the processed contents is due to the high heat resistance of the ascospores. The original infection is probably introduced into the can on the fruit, as ascospores were detected on raw fruit in orchards and in the factory by heating the material before plating it out; in this way mould spores were killed but *B. fulva* ascospores remained viable. Olliver & Rendle's experiments showed that a suspension of ascospores in fruit syrup withstands a temperature of 86–88° C. for 30 min., and when the suspension is heated at a similar rate to that at which the contents of a can are heated during processing, viable spores are present until a temperature of 98° C. is reached. This temperature is considerably higher than that attained during normal processing of fruit.

Hirst & McMaster (1933) gave the history of the occurrence of the trouble as experienced at the Fruit and Vegetable Preservation Research Station, Campden. They first encountered it in 1931, but did not associate it with *B. fulva*: a small disk of mycelium was often seen amongst the disintegrated contents of the can, but it would not grow in culture and appeared to be dead.

In subsequent years other instances were reported by canners from most parts of the country so that the trouble appeared to be widespread. On the other hand observations at the Research Station suggested that severe fruit infection might be confined to certain orchards. For two consecutive years all the fruit (strawberries) from a certain grower gave a badly infected product both at the Research Station and at a nearby

factory, whereas similarly processed fruit from other sources behaved satisfactorily. This observation suggested that heavy infection might be confined to certain orchards, so that it was desirable to investigate the occurrence of the fungus in the field. Also, from the growers' point of view, it was desirable to know more about the field occurrence, as those growers whose fruit had once given an infected product had difficulty afterwards in selling their fruit to canners.

Hirst & McMaster (1933) described experiments in which inoculated cans were processed for various times, and which suggested that the ascospores might be killed during processing in some of the hard fruits, but not in soft fruits. Even in the hard fruits, where the mould was killed, the temperature attained in the can was only between 87–91° C., which was considerably below that (98° C.) at which Olliver & Rendle had found the spores to remain viable in their experiments. Thus it was possible that there was some factor which made sterilization in the can easier than was indicated by laboratory results.

The work described in this paper was undertaken to determine to what extent the fungus was disseminated in the field, whether any field control was practicable, and to what extent control was possible during the normal canning process. Progress reports containing some of the results included in this paper were published during the investigation (Hull, 1934, 1935).

II. Occurrence in the field

A method of isolation which took advantage of the heat resistance of the ascospores of *B. fulva* was used to determine whether spores were present on material from the field. Samples of leaves, fruit, straw, etc. were collected in plugged sterile tubes of appropriate size. These were poured in the laboratory with hot (*c.* 60° C.) potato-sucrose-agar, acidified with hydrochloric acid till it would just set, and while the agar was still liquid the tubes were heated in a water-bath at 80° C. for 30 min., sloped, and incubated at 30° C. when they had set. This procedure was found by experiment to give the maximum count of samples showing *B. fulva*; shorter heatings yielded tubes with many other micro-organisms and these prevented *B. fulva* from growing.

Samples were collected and treated in this way from a strawberry plantation at Campden the fruit of which had given an infected "pack" in the previous year. A few samples of dead leaves were taken in January, 1933, and some positive results were obtained. In April both young and

old leaves collected in the field yielded the fungus in 33 of the 100 samples taken and numerous subsequent samplings from the same plantation gave similar results. Ripe berries sampled in the field in the middle of July showed 26 % contamination.

Strawberry plantations in Kent were visited in June and large numbers of leaf samples were collected. Contamination varied somewhat in different districts. Thus two fields near Maidstone showed 6 and 13 % respectively; two fields at Barming showed none in 40 samples taken from each; fields at Yalding and Goudhurst gave 25 and 20 % respectively; four fields near Faversham each gave 5–8 %, and one field at Swanley showed 7 %. Samples of the fruit which had been obtained from these plantations were collected at the canning factories and showed the presence of *B. fulva* spores. Samples of leaves taken in April from various fruit trees in orchards near Colchester were found to be slightly contaminated. In certain plantations in Gloucestershire of raspberries, loganberries, black-currants, gooseberries and plums, fruit and leaf samples were collected at various times, and contamination was found in all cases. Samples of fruit, usually 50–100 berries, were collected throughout the season on arrival at the Research Station, Campden, at factories and in orchards, and varying amounts of contamination were found. Further reference to the extent of contamination of the fruits is made in Table X.

No fungal growth was observed on the samples as collected, and the fact that growth of *B. fulva* usually started from one centre suggested that occasional ascospores had lodged on the sample. Evidence bearing on the source of these spores was obtained from the following observations. Runners from strawberry plants in the fields at Campden had been planted during the previous year in an isolated field about 10 miles away. There were no other orchards within half a mile of this field, the surroundings being pasture and plough-land. Samples of leaves taken from here at various times showed 5 10 % contamination as compared with 15–20 % for runners taken from the same stock and planted near the original field at Campden. This suggested that there was some source in the Campden district which was emitting spores into the air near the fruit plantations.

It was found that mummified plums, taken in the winter from an orchard close to the strawberry fields, were heavily contaminated with *B. fulva* ascospores. In cultures prepared from such plums, fungal growth began at numerous centres in the agar slope, whereas, in similar preparations from leaves, growth usually took place from one centre only.

It was suggested that the ascospores had been produced on the mummified plums and from them had reached the leaves.[1]

Field evidence in support of this view was obtained in a raspberry plantation at Evesham, in which the canes were planted under plum trees. In one half of this orchard the trees were old and carried numerous mummified plums, in the other the trees were young and had few mummified plums. Leaves from raspberry plants in the two halves showed 34 and 18 % contamination respectively.

Fruit refuse, such as "second crop" raspberries which had been left on the canes, was found in some cases to be heavily contaminated after the manner of mummified plums.

A secondary source of contamination was established in one case where old chip baskets which had been used the previous season were being used again after storage. Remains of fruit present on the linings were heavily contaminated with *B. fulva* ascospores, which had been produced *in situ*.

It was confirmed that *B. fulva* does not parasitize living fruit tissues. Fruits killed by heating at 100° C. for a few minutes supported prolific and rapid growth when incubated at 25° C. Attempts were made to grow *B. fulva* on fruits which had been overrun by parasitic fungi, but with only slight success. Dry mummified plums do not support growth, but in some cases slight growth was obtained when they were moistened, although often the growth of other fungi predominated and that of *B. fulva* could not be detected. *B. fulva* grew very prolifically on autoclaved mummified plums.

Samples of straw taken from a strawberry field a few days after it had been laid showed slight contamination but less than that obtained from leaf samples gathered at the same time; thus it was not likely that infection was brought into the field on the straw.

No support was found for the suggestion of Olliver & Rendle (1934) that the fungus might be a soil organism. It did not grow on sterilized or unsterilized soil. Fruits picked from the ground showed no greater contamination with *B. fulva* than others gathered from the trees above.

The foregoing observations indicate that the natural substrate of the fungus is fruit refuse, more particularly mummified plums. In view of the high temperature at which the fungus grows and produces asci it may be assumed that growth and ascus formation takes place during the

[1] Attempts to demonstrate microscopically the presence of mycelium or ascospores of *B. fulva* in surface scrapings of mummified plums were unsuccessful, possibly because of the large amount of fungal spores (*Monilia*, etc.) and debris present.

summer months when fruit refuse is abundant, and that later the ascospores are scattered indiscriminately over the neighbouring vegetation by air dissemination.

III. Physiological characteristics

(*a*) *Methods and materials*

Throughout the investigation frequent isolations were made from affected cans and bottles of fruit, and when the fungus developed it was invariably found to be in pure culture. Occasionally no growth resulted but the presence of the fungus could be confirmed by finding hyphae or a disk of mycelium amongst the disintegrated contents. Cans inoculated with ascospores of the fungus and subjected to the normal factory process showed the characteristic symptoms of disintegration of the fruit tissues. Reisolation gave pure cultures of the fungus; this was repeated frequently and left no doubt that the fungus was the cause of the spoilage.

Isolations were made from a suspected can or bottle by withdrawing a 5–10 c.c. sample under aseptic conditions and placing it in a sterile Petri dish where it was mixed with either nutrient or plain agar, or in a tube, in which it was incubated in the liquid state. If the fungus was present growth became visible within 36 hr. at 30° C. and its identity could be recognized by the characteristic buff-coloured conidial structure. For confirmation, the cultures were occasionally kept longer until asci were produced.

Freshly isolated cultures produce asci readily on potato-dextrose-agar and other plant-extract media, but less profusely on the usual synthetic media. At 30° C. the beginnings of ascus formation are visible in 4–5 days and mature ascospores are present after about 10 days. At 25, 20 and 15° C. ascospores could be found on plates after 8, 19 and 42 days respectively. The rate of formation of ascospores is, therefore, relatively slow at ordinary summer temperatures so that there is no risk of their increasing greatly in number during the time the fruit is being handled. Although an isolate gave satisfactory ascus production at first, after subculturing several times during 4 months it gave colonies which were covered with a snow-white fluffy mycelium. This became a tough mass on the surface of the agar and produced very few or no ascospores, so that new cultures for ascospore production had then to be obtained from affected cans.

The asci could easily be picked up in masses from the surface of the agar with a needle, and they produced a uniform suspension when shaken with water. As the ascus usually remained intact even after the suspension had been filtered, centrifuged and washed, it was the germination of the ascospores while still in the ascus which was studied and all the counts mentioned later deal with asci and not ascospores. On germination one or more ascospores develop a swelling at one end, as large as the spore itself, from which one or more germ tubes grow. The ascospores remain together after germination so that an ascus with germinated spores appears large and is thus easily identified, even if the asci are somewhat clustered together.

Germination studies were carried out by spreading a suspension of asci of suitable density over the surface of plain or nutrient agar plates, acidified with 0·5% malic acid, and incubating at 30° C.

Determinations of the thermal resistance of spores were made by adding a dense suspension of asci, prepared by filtering cultures through muslin and centrifuging, to 10–20 volumes of water heated to the desired temperature in a thermostatically controlled water-bath. Samples were then removed from time to time for germination studies.

(*b*) *Thermal relations of spores*

Unheated ascospores when placed on agar either do not germinate or do so sporadically; after heating to a certain degree, quick and regular germination is obtained. A similar phenomenon was reported by Gwynne-Vaughan & Williamson (1930) when young ascospores of *Humaria granulata* failed to germinate unless heated. The effect on the germination of asci of *B. fulva* of heating in distilled water at temperatures between 65 and 85° C. is shown in Table I.

Table I. *Percentage germination of asci after heating*

	% germination after four intervals (hr.)			
Temp. and time of heating	14	21	25·5	38
85° C. 10 min.	0	0	+	38
30 ,,	0	0	0	1
80° C. 10 min.	+	96	95	Weft
30 ,,	+	70	72	,,
75° C. 10 min.	+	99	98	Weft
30 ,,	+	93	96	,,
70° C. 10 min.	+	28	41	Weft
30 ,,	+	49	51	,,
65° C. 10 min.	0	0	0	5
30 ,,	0	0	2	3
Unheated	0	0	0	<1

+ Germination just beginning.

It is clear from Table I that optimum germination is obtained by heating the spores for 10 min. at 75–80° C. or for 30 min. at 75° C.

Fig. 1 shows the effect on germination of various periods of heating at 70 and 85° C.

Heating at 70° C. stimulated germination, and the effect was correspondingly greater the longer the time of heating, up to the maximum used in the experiment. This curve represents the germination which had taken place after 15 hr. incubation. With further incubation the percentage germination increased on all plates and was probably potentially 100% in all cases, though this could not be observed in practice as the mycelial growth from the quickly germinating spores smothered the plates. At 85° C. a short exposure (*c.* 1 min.) gave 100% germination but the percentage germination decreased rapidly with longer exposures. A large proportion, approximately 90% of the asci,

were killed relatively easily, but many of the last 10% had a much greater heat resistance.

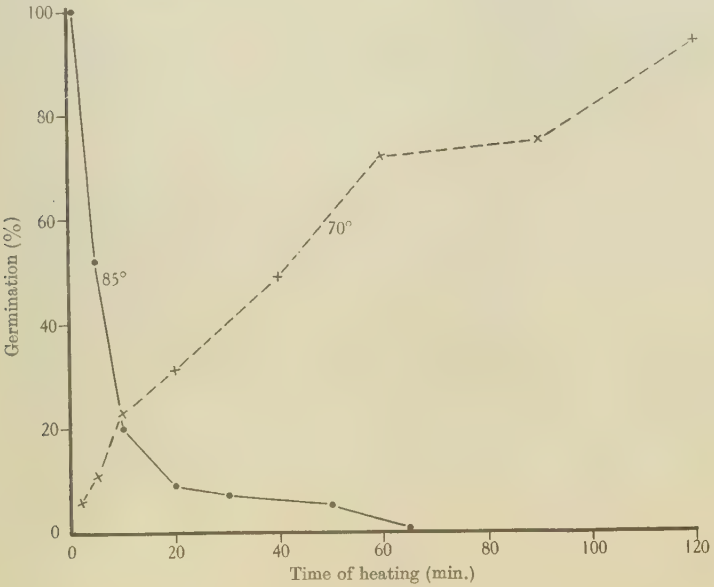

Fig. 1. Percentage germination of spores after being heated for various times at 70 and 85° C.

Determinations of the lengths of exposure which were necessary at various temperatures for killing all spores gave somewhat irregular results so that the figures obtained from any one experiment cannot be relied upon. Table II gives the *maximum* time of resistance in distilled water for the given temperature, as obtained in a number of experiments.

Table II. *Heat resistance of spores*

Temp. of heating (° C.)	94	92	90	88	86
Time above which no growth was obtained (min.)	2	5	20	40	100

A comparison of Table II with Fig. 1 shows that while the majority of spores are killed by a short exposure to 85° C., long times are required at even higher temperatures to ensure complete sterilization. An important conclusion derived from these experiments is that a small fall in temperature necessitates a great increase in the time of heating necessary to kill *all* the spores.

Henderson Smith (1923), working with spores of *Botrytis cinerea*, found that the curve representing the percentage of spores surviving various times of heating between 37–50·3° C. is of sigmoid nature. The amount of variation of heat resistance between individual spores is less than that of *Byssochlamys fulva* ascospores, but as with *B. fulva* the temperature coefficients for the reaction of killing *Botrytis* spores by heat are very high.

The heat resistance of *Byssochlamys fulva* ascospores was also determined under conditions of gradually increasing temperature. A heavy suspension of ascospores in distilled water was heated in a water-bath and samples were pipetted out at intervals and placed in "medical flats" containing apple extract solution. The temperature of the suspension when the sample was removed, and the time taken to attain that temperature, were recorded. The medical flats were incubated for 4 days and then the dry weight of the mycelium which developed, which was a function of the number of spores surviving, was determined. The results are shown in Table III.

Table III. *Growth obtained from heated ascospores*

Time of heating min.	Temp. °C.	Mycelium weight g.
4	85·0	0·275
5	89·5	0·282
6	92·5	0·203
7	94·0	0·009
8	95·0	0·001
9	96·0	Nil

Thus, heating above 89·5° C. reduced the amount of mycelium which was produced, and none was obtained after heating to 96° C., even when the samples were incubated for a longer period. At 96° C. all the spores were killed, so this temperature is the approximate thermal death point of the ascospores, although the value would vary somewhat according to the rate at which the temperature was raised.

Heat resistance of different isolates. Ascospores from different isolates behave differently on heating. Cultures isolated from different sources were grown under similar conditions and ascus suspensions from each were heated for various times at 85° C. Table IV gives the percentage germination after 10 min. heating. Confirmatory data were obtained for other periods of heating.

Age of culture and heat resistance. Ascospores of ages varying from $3\frac{1}{2}$ to 30 weeks were obtained from cultures which had been maintained at 30° C. The cultures were isolated from infected cans which had been

inoculated with the same stock culture. Suspensions of these ascospores when similarly heated gave germination figures ranging from 2 to 69%, but this variation was not obviously correlated with the ages of the cultures.

Table IV. *Percentage germination of asci after heating*

	% germination Time of incubation (hr.)	
Origin of culture	26	45
From a can; subcultured for some time	2	38
From orchard, Campden	11	32
From infected strawberries canned at Evesham	30	Weft
From infected strawberries canned at Whitchurch	54	Weft
From field, Evesham	91	Weft
From field, Kent	0	0
From field, Colchester	84	Weft

Effect of reaction of heating medium on heat resistance. The heat resistance of spores is influenced to some extent by the reaction of the medium in which they are heated. Fig. 2 gives the average results of several experiments in which spores were heated in buffer solutions covering a range of *p*H values.

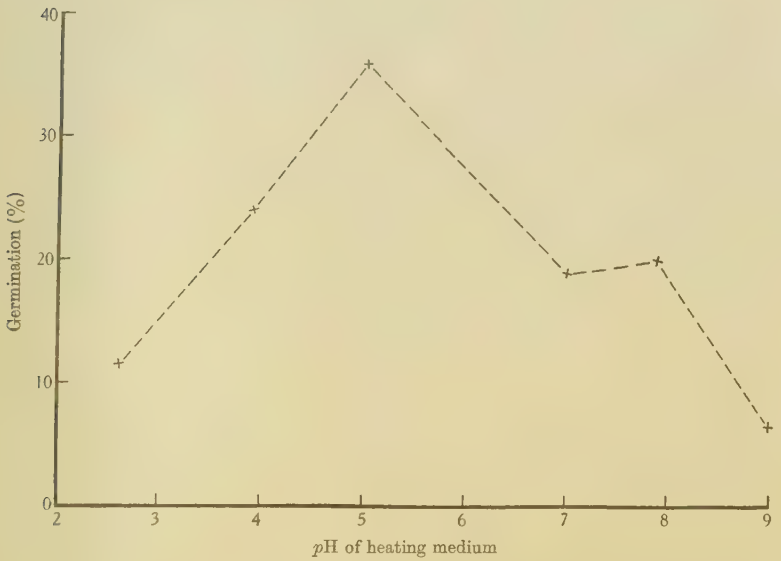

Fig. 2. Percentage germination of spores heated in solutions of various *p*H values.

Heat resistance was greatest at about *p*H 5. It is interesting to note that in the canning process the *p*H of the syrup taken at the middle of the "cook" varies from *p*H 2·9 in loganberries to *p*H 3·8 in strawberries (Adam, 1934).

Effect of concentration of sucrose in heating medium on heat resistance. The concentration of sucrose in the solution in which asci were suspended had a considerable effect on the heat resistance of the asci. Table V shows the percentage germination, after 40 hr. incubation, of asci which have been treated in various concentrations of sucrose and then placed on plates of plain agar.

Table V. *Percentage germination of asci heated in sucrose solutions*

	% germination Time of heating at 84–5° C. (min.)			
% sucrose	5	10	20	30
0	Weft	65	21	1
5	,,	62	5	1
10	,,	77	16	11
30	,,	Weft	37	15
65	,,	,,	Weft	75

Increasing the sugar concentration had a protective action on the asci, rendering them more resistant to heat treatment. In this respect Baumgartner & Wallace (1934) found that sucrose concentrations between 10 and 30 % had a similar protective action on vegetative cells of *B. coli*, but up to 50 % had no effect on bacterial spores or strains of *Torula*.

Heat resistance and germination of conidia. Whereas ascospores showed a remarkable heat resistance, conidia were killed relatively easily. All the spores in a suspension of conidia were killed after heating for 10 min. at 70° C.: heating for 15 min. at 51·5° C. reduced the germination to under 10 % but the remainder were not killed after 30 min. at this temperature. Unheated conidia germinated in apple extract at 37° C. within 4 hr. and were reproducing conidia by this time.

(*c*) *Factors influencing ascospore germination and mycelial growth*

Temperature. The following data illustrate the effect of the temperature of incubation on the germination of pre-heated (75° C. for 10 min.) ascospores. At 38 and 32° C. germination began after 10 and 15 hr. respectively, the majority of the asci germinating simultaneously and the remainder shortly afterwards. At 25 and 20° C. germination had begun within 24 and 43 hr. respectively, but ungerminated asci were

seen in the tangle of mycelium long after this. At 15° C. there was no germination in 87 hr.

Radial growth on potato-dextrose-agar was fairly rapid at 35° C., amounting to 3·6 cm. in 3 days. With lower temperatures it diminished progressively and at 15° C. was very slow. At 8° C. there was no growth.

The dry weight of mycelium produced in Richards' solution after 5 days' incubation at various temperatures is shown in Table VI.

Table VI. *Dry weight of mycelium at various temperatures*

Incubation temp. (°C.) ...	20	26	28	32	41
Mycelium dry wt. (g.)	0·140	0·436	0·648	0·999	0·400

The optimum temperature for ascospore germination and mycelial growth thus lies in the neighbourhood of 35° C.

Reaction of medium. Ascospores were placed on plates of potato-extract-agar adjusted to *p*H values between 1·8 and 8·0. The ascospores failed to germinate at *p*H 1·8 but germinated on all the other plates; the most rapid germination and growth took place at *p*H 3·5. This is close to the value (*c.* *p*H 5) at which the maximum heat resistance of ascospores was obtained.

Sucrose concentration of medium. Both ascospore germination and mycelium growth took place in high sucrose concentrations. Asci, previously heated to 75° C. for 20 min. to ensure even germination, were placed on agar plates containing various concentrations of sucrose up to 70%. Germination was observed within 18 hr. on the plates having sucrose concentrations up to 50%, and on the remainder after 37 hr. It was more rapid on the 20% than either the 0 or the 40% plates. All plates eventually showed 100% germination, so that the main effect of sucrose concentration greater than 20% was to delay germination. In the higher concentrations of sugar the germ tubes of the spores were numerous and fine.

When heated spores were grown in apple extract solution containing added sucrose up to 65%, diminished weight of mycelium was obtained with each successively higher sugar concentration, but even at an added concentration of 65% growth still occurred. The sucrose concentrations in cans of fruit are usually about 30%, and these would not cause any pronounced depression of mycelial growth.

Antiseptics. The asci showed remarkable resistance to antiseptics. A suspension of spores was immersed in solutions of various antiseptics, centrifuged after a given time and the spores washed in sterile distilled water. Growth was obtained after immersion in 10% formaldehyde for

10 min., in 10 % "Lysol" for 30 min., in 0·5 % sodium hypochlorite for 10 min. and in 0·5 % mercuric chloride for 36 min.

Carbon dioxide concentration. In a later section the growth of the fungus in concentrations of carbon dioxide of its own production up to 65 % will be described. In an experiment in which ascospores were placed on plates of potato-sucrose-agar and in apple extract in atmospheres with different concentrations of carbon dioxide, diminished growth and much retarded germination were obtained at concentrations above 60 %. The fungus is thus relatively insensitive to carbon dioxide.

Desiccation. Ascospores are not easily killed by desiccation, as tube cultures which had been kept in a dried condition for 4 years produced growth on subculturing.

(*d*) *Enzyme production*

Growth is accompanied by the production of a pectin-destroying enzyme, which causes the breakdown of the fruit tissues. The enzyme was detected by the softening of potato disks in solutions in which the fungus had been growing, but the action was slow. Concentrated enzyme extracts were prepared by germinating ascospores in medical "flats" at 30° C., collecting the resulting mycelium while very young and following the usual method of enzyme extraction (Brown, 1915). The enzyme extract thus obtained rapidly disintegrated potato disks and the tissues of canned fruits.

IV. Canning experiments

(*a*) *Methods and materials*

Various modifications of the fundamental canning process are used in practice. Briefly, the process is as follows. The fruit is prepared and filled into the cans and is covered with a warm sugar solution to within about $\frac{1}{4}$ in. of the top of the container. The cans are then placed in hot water to remove air trapped amongst the fruits; this is called the "exhaust" process. The lids are seamed on and the cans placed in water, usually at boiling point, for the "cooking" process. After the appropriate time the cans are removed and cooled. As the lids are sealed on when the contents are hot, the internal pressure is reduced on cooling and the reduction below atmospheric pressure is called the "vacuum".

In all the inoculation experiments in cans performed at Campden the procedure was standardized as much as possible. The cans used were of size A 2. The syrup had a density of 45° Brix[1] unless otherwise stated; it was filled at 170° F.[1] by hand. The cans were exhausted in a bath at 185° F. for 6 min. and the "cooks" were made in

[1] As in canneries, the Brix hydrometer was used for measuring syrup densities. It gives a direct reading of the percentage of sugar by weight in the solution. The Fahrenheit temperature scale is also employed in canneries and so is used in this section.

boiling water. Where possible the cans were kept stationary in the cooking bath, but in some of the soft fruits a sufficiently high internal temperature could not be attained by this method, so the cans were laid on their sides in wire trays and rolled gently at 2 min. intervals. This method is referred to as a "rolling cook" in contrast to the former which is called a "stationary cook". A "rotary cook" refers to the mechanical process normally used in factories.

Inoculations of three densities of spore suspension were used. A spore suspension was made in distilled water from a culture about 3 months old. The suspension was filtered through muslin and centrifuged. To each 0·1 c.c. of centrifuged spores, 10 c.c. of sterile water were added, and the spores were shaken into an even suspension. This constituted the "heavy inoculum" used in the experiments, and 1 c.c. of this suspension was added to each can. The "standard inoculum" was this suspension diluted $\times 10$, and the "light inoculum" was the "heavy inoculum" diluted $\times 1000$. A count of the "light inoculum" showed the presence of approximately 100 spores/c.c., but owing to the method of making the spore suspension, this figure was not taken as absolute, but rather as showing the order of the number of spores. Thus, there would be approximately 10^5 spores in the cans with heavy inoculum, 10^4 with standard and 10^2 with light inoculum. In all cases the cans were inoculated after the fruit had been inserted but before adding the syrup.

The fruit used in the experiments was obtained from local growers. While it was being picked and placed in the cans, samples were also placed in sterile tubes by the pickers, and were examined by the method already described for the presence of ascospores of *Byssochlamys fulva*. Any inoculum added to a can was additional to that naturally introduced on the fruit. The fruit in control cans contained the natural infection only.

After processing, the cans were stored at room temperature or at 37° C. if they were to be examined within a fortnight. In any case they were always given a period of 3–7 days at 37° C. before examination. They were opened under aseptic conditions and a sample of their contents examined for the presence of *B. fulva*. In each case two samples (approx. 5 c.c. each) were placed in Petri dishes and poured with agar, and one tube sample (approx. 10 c.c.) was kept. The texture of the fruit was noted so that had the fungus grown and subsequently died a deceptive result would not be obtained.

Two methods of measuring the internal temperature of the contents of a can during the cooking process were used. When the cans were heated in a stationary tank lids pierced with a large hole were used, and after the lids had been seamed on, a thermometer passed through a rubber stopper was inserted tightly in the hole. The length was such that the bulb reached to the centre of the can. The can was then placed in the boiling tank and the rise in temperature at the centre of the contents recorded. When the cans were rolled or extracted from a rotary cooker, the piercing armoured thermometer was used. This had a base armoured with a pointed metal case which was plunged into the end of the can to a depth defined by the position of a stopper on its stem. The thermometer had a considerable heat capacity, so before use it was heated approximately to the temperature expected to be recorded.

Although the results obtained on different cans with one instrument were comparable, those obtained with the different instruments were not. This was because the fixed thermometer was inserted in the syrup between the fruits, whereas the piercing thermometer took an average record of the temperature of the syrup and that of any

fruit which it happened to pierce. Also, syrup often spurted out of the can when the end was pierced with the thermometer, and this would involve some movement of the contents which normally would not take place. However, as the instrument was used mainly for recording temperatures in agitated cans, the error caused by this spurting was not so serious as it would have been in the case of stationary cans.

The general results obtained were that a can agitated in the cooking bath heated much more quickly than a stationary one, and that with a stronger syrup the contents heated more slowly than with a weaker syrup. Apart from these variations with any one fruit, soft fruits such as strawberries and raspberries heated much more slowly than smooth hard fruits such as gooseberries and plums.

(*b*) *Experimental results*

Inoculations with standard suspension of ascospores. Cans of golden plums were inoculated with the *standard* suspension and cooked for various times. The amount of infection obtained is shown in Table VII.

Table VII. *Infection in inoculated cans of golden plums*

Time of "cook" (min.) ...	12	16	20	24
Internal temperature (°F.) ...	181·5	189·3	191·5	193·3
Proportion of cans infected* ...	5/5	4/5	0/5	0/5

* The numerator shows the number of cans infected and the denominator the number of cans used.

When the time of "cook" was longer, and consequently the internal temperature higher, the proportion of infected cans was reduced. This result is typical of a series of experiments with various fruits; the details of the times of "cook" necessary to produce sterility in all the cans used and the internal temperature recorded for that time are given in Table VIII.

Table VIII. *Sterility in inoculated cans produced by heat treatment*

	Time of "cook" min.	Internal temp. °F.
	Stationary	
Black currants	20	195·2
Victoria plums	24	188·0
Damsons	20	189·7
Blackberries	24	189·2
Cherries	8	200·2
Golden plums	20	191·5
Gooseberries	20	195·3
	Rolling	
Raspberries	16	194·5
Strawberries	17	188·0
Loganberries	16	200·5

The internal temperatures necessary for sterilization varied between 188 and 201° F., but as the different "cooks" used were at 4 min. intervals the actual lethal temperature might be somewhat lower than that recorded. With cherries, which heat relatively quickly, the lethal temperature was attained rapidly, but with the other berries a "stationary cook" of 20–24 min. was necessary. With the softer fruits, for which a "rolling cook" was used, the lethal temperature was obtained in 16–17 min. In most cases, the times of cooking necessary to produce these fully lethal temperatures are considerably longer than those normally used in canning practice.

Further determinations of the lethal conditions were made in factory rotary cookers. For instance, size A 1 tall cans, filled with loganberries, were inoculated with a standard suspension of spores and were collected at each of the four doors of the cooker. The 3 cans from the second door, which received a "cook" of 4 min. and showed an internal temperature of 191° F., remained infected, but the 5 cans from the third door, which received a "cook" of 5½ min. and showed an internal temperature of 199° F., were sterile.

Variation in amount of inoculum. The results of the inoculation of gooseberries, loganberries and plums with three different concentrations of spore suspension are shown in Table IX.

Table IX. *Infection in cans inoculated with different concentrations of spore suspension*

Time of "cook" (min.) ...	8	12	16	20
	Proportion of infected cans			
Gooseberries				
Light inoculation	3/5	3/5	0/4	0/4
Standard inoculation	5/5	5/5	3/4	0/4
Heavy inoculation	3/5	5/5	2/4	0/4
Loganberries				
Light inoculation	4/4	0/4	0/4	0/4
Standard inoculation	4/4	2/4	0/4	0/4
Heavy inoculation	4/4	1/4	0/4	0/4
Golden plums				
Light inoculation	5/5	1/5	0/5	0/5
Standard inoculation	5/5	4/5	0/5	0/5
Heavy inoculation	2/5*	2/5	1/5	1/5

* These cans were opened 8 months later and there were signs of slight disintegration in some berries. Thus infection may have occurred, but the mycelium died before the cans were examined.

With the light inoculum the cans were more easily sterilized than with the heavy or standard inoculum. This was in agreement with the observation that only a small proportion of the spores have a high heat

resistance; with the light suspension there would be less chance of the presence of very resistant spores in the can. The fact that there were only slight differences in the time necessary to produce sterility between the standard and heavy inocula suggested that these suspensions were sufficiently concentrated to ensure the presence of the most resistant spores. Therefore, the canning results for these inocula give a time of "cook" which is the maximum necessary to overcome any infection.

Natural infection. The method of detecting the presence of *B. fulva* spores on the fruit has been described above, and the reasons given for the view that the spores were present there in small numbers only. Assuming each infected fruit to bear only one spore, the approximate amount of natural infection entering the can may be estimated. This is included in Table X together with the data gained from canning experiments concerning the nature of the "cook" necessary to overcome this natural infection.

Table X. *Sterility in cans of naturally infected fruit produced by heat treatment*

Fruit	% infection of berries	Approx. no. of fruits in A2 can	Estimated no. of spores in each can	Time of "cook" for sterility min.	Internal temperature in can °F.
		Stationary			
Victoria plums	26	12	3	12	178·5
Blackberries	16	80	13	20	184·7
Golden plums	10	12	1	12	181·5
Gooseberries	57	63	36	12	190·0
Black currants	29*	900	260	12	191·2
		Rolling			
Blackberries	16	80	13	8	190
Raspberries	57	160	91	6	185
Loganberries	28	80	22	8	193
Strawberries	8	40	3	10	171
,,	36	40	14	5/7 infected after 9 min.	176

* Several berries were placed in each tube in the case of black currants.

The natural infection introduced fewer spores into the can than did the suspensions used in the inoculation experiments. The highest temperature required to overcome the natural infection in all the cans used was 193° F. and a lower temperature was enough in most cases. On the other hand, when strawberries bearing considerable natural infection were given the "cook" normally used (9 min.) 5 out of 7 cans remained infected.

A few experiments were performed in factories when the fruit was sampled before filling the cans and the "central" temperature attained

in the cans during processing was recorded. Cans selected at random from the final product were examined for the sterility of their contents. For instance, gallon cans of golden plums in water attaining a "central" temperature of 185° F. (stationary cook) were sterile, although the fruit when sampled at the time of filling the cans showed 20 % of the berries infected. However, cans inoculated with the spore suspension and given the same treatment remained infected. In another case, golden plums, of which 15 % of the berries were infected, were filled into size A $2\frac{1}{2}$ cans and these were removed from the doors of a rotary cooker. Five cans from the first door (cook $2\frac{3}{4}$ min.) gave a central temperature of 184° F. and all were sterile.

Inoculation into different strengths of syrup. The laboratory experiments showed that a higher sucrose concentration increased the heat resistance of ascospores. Also, various physical properties of stronger sugar solutions lead to a slower rate of heating. It was proved by experiments, the results of which are shown in Table XI, that times of cooking sufficient to sterilize in 45° Bx syrup would not do so in 55° Bx syrup.

Table XI. *Effect of density of syrup on the proportion of infected cans*

	Time of "cook" min.	45° syrup		55° syrup	
		Temp. °F.	Infection	Temp. °F.	Infection
		Stationary			
Gooseberries	20	195·3	0/4	185·0	3/3
Golden plums	20	191·5	0/5	188·2	1/5
		Rolling			
Raspberries	14	193·0	0/4	—	4/4

In such canning experiments it is not possible to separate the effect of increased heat resistance of the spores from decreased temperatures in the can and, therefore, no conclusions were reached as to the relative importance of the two factors, but the experiments showed that the combined effects were of importance in practice.

Effect of time of cooking on the texture of the fruit. As the times of cooking necessary to overcome infection with *B. fulva* were longer than those normally used, preliminary experiments on the effect of longer cooking on the final appearance of the fruit were made. This effect would be much influenced by the variety of fruit and its condition of ripeness and freshness. Such experiments were made on only one batch of each fruit, but all the fruit used was of a satisfactory state of ripeness, except the raspberries and blackberries, which were slightly overripe.

Cans were treated in the normal way and given different times of cooking, 3 cans being used each time. After being stored for 1–3 months they were opened and examined by the method used for assessing the quality of canned fruit (Hirst & Adam, 1932). In plums and soft fruits the "cook" necessary to overcome the standard inoculum softened the fruit. This softening was, however, not nearly so serious as that caused by growth of the fungus. The texture of the fruit was undamaged by cooks which were sufficient to overcome the natural infection. The harder fruits were improved by longer cooking and there was no difficulty in their sterilization.

The flavour of the soft fruits deteriorated after cooking for a time which softened the berries, but no difference in flavour was detected for different times of cooking for the hard berries. The times of cooking did not affect the colour of the fruit. Raspberries, loganberries and blackberries showed a diminishing "drained weight" of the fruit with increasing times of cooking, which suggests that the fruit had shrivelled.

(*c*) *Degree of control obtained*

To obtain sterility in heavily inoculated cans, which contained some of the most resistant spores, long heatings which gave an internal temperature of 190 200° F. were necessary; such heating tended to damage the texture of the fruit. With a smaller artificial infection, so that there was less chance of the presence of very resistant spores, a smaller proportion of cans remained infected after a given time of cooking. The natural infection on the berries invariably introduced many fewer spores into the container than were introduced by inoculation, and this natural infection could be overcome by a time of cooking which was not detrimental to the flavour and texture of the fruits tested. However, although only a very small proportion of the spores are highly heat resistant, there is always the chance that such a spore is present, and so long as a temperature of approximately 195° F., at which all the spores are killed, is not attained, there is the possibility of infection in an occasional can.

Spoilage in individual cans of a batch which has not received a heating which achieves this totally lethal internal temperature depends upon three factors: (*a*) the occurrence of a spore in the can which resists the heating given; (*b*) whether the spore happens to be situated in the can in a position where a high temperature is attained; (*c*) upon the packing of the fruit, which affects the temperature attained in the individual can. It is a matter of chance whether these factors coincide or not, consequently leaving the can sterile or infected.

During the 1934 season, when suggested control measures were circulated to canners, some 350 representative samples of canned fruits from factories all over the country were examined for *B. fulva*. Infection was found in 18 cans which came from only two factories. The cans contained raspberries, strawberries and loganberries—fruits for which it is most difficult to devise a satisfactory internal temperature. Samples of these fruits from other factories were sound, so that the majority of factories were obtaining good control.

(*d*) *Mycelial growth in cans*

Experiments showed that no growth of *B. fulva* develops in an atmosphere from which oxygen has been removed by alkaline pyrogallol; therefore the amount of mycelial growth which develops in an infected can depends upon the amount of oxygen available. The original amount of oxygen present is dependent upon such factors as size of headspace, type of fruit and temperature of sealing. Oxygen is used by the fungus, and also by chemical action (Horner, 1934), so that the amount of mycelium which develops depends on the relative rate at which oxygen is used by chemical action and fungal metabolism. The residual fungal infection in the can is also a factor determining the weight of mycelium produced. With a heavy infection the absolute increase of weight of growth is greater in a given time than with a light infection, and so the fungus absorbs oxygen which would otherwise be used in chemical reaction. This accounts for what is often observed in practice, that mycelial development, and hence the extent to which the fruit is softened, varies considerably in individual cans.

The change in composition of the headspace gas in control and infected cans of Pershore plums stored at 30° C. was determined. The headspace was 28–33 c.c. The gas compositions[1] are shown in Table XII.

Table XII. *Composition of gas in cans*

Time of storage days	Control		Infected	
	CO_2 (%)	O_2 (%)	CO_2 (%)	O_2 (%)
2	15·0	14·0	17·5	10·5
3	—	—	26·5	4·5
4	—	—	38·0	1·0
6	19·0	5·0	45·0	0·0
13	21·0	0·0	52·0	0·0
22	22·0	0·0	—	—

[1] These gas analyses, and others mentioned later, were made by Mr G. Horner with his apparatus (1934).

In infected cans the carbon dioxide concentration increased to 52% and this was accompanied by a gradual reduction of the oxygen concentration to zero. In the control cans there was a slight increase in carbon dioxide concentration on storage and a reduction in oxygen concentration, but at a much slower rate than in the infected cans.

In all cases where series of infected and uninfected cans had received similar treatment, and had been examined at the same time, a reduction in the vacuum of the infected cans was observed. In some cases the affected cans were "blown" while the uninfected cans retained a good vacuum. This change in pressure is due to production of carbon dioxide by the fungus and indicates that a form of anaerobic respiration takes place. Some of the factors affecting the change in gas pressure and the development of mycelium are described below.

Effect of headspace. Series of cans, containing plum syrup but no fruit, were arranged so that they had different headspaces. They were inoculated with spore suspension and put through the normal process, being cooked for 5 min. at 80° C. After the cans had been incubated 9 days at 37° C. a determination was made of the pressure and composition of the headspace gas and the weight of mycelium produced. The results are shown in Table XIII.

Table XIII. *Relationship between headspace and growth of mycelium in cans*

Filling (c.c.)	560	530	500	450
Headspace (c.c. approx.)	25	55	85	135
Pressure (cm. variation from 76 cm. Hg)	−24·8	7·1	18·1	26·1
CO_2 in headspace gas (%)	43·6	57·4	64·4	63·9
Dry weight of mycelium (g.)	0·032	0·056	0·072	0·091

The amount of mycelial growth was greater with increasing headspace. Greater mycelial growth was accompanied by more carbon dioxide production, and thus a greater gas pressure was generated in the container.

The results in Tables XII and XIII suggest that it is depletion of oxygen rather than the production of an inhibiting concentration of carbon dioxide which normally limits growth of mycelium in the can. Lack of oxygen is probably the cause of the eventual death of the mycelium.

Effect of storage temperature. Cans were filled with Richards' solution, acidified to *p*H 3 with citric acid, and it was arranged that two volumes of headspace were obtained, the one approximately 50 c.c. more than the other. The cans were inoculated with *B. fulva* ascospores and put through

the normal process, being closed at 77° C. and cooked for 10 min. at 85° C. They were then stored at different temperatures and determinations were made at intervals on 4 cans for each treatment. The data obtained are shown in Table XIV.

Table XIV. *Relationship between storage temperature and growth of mycelium in cans*

Storage temp. °C.	Time of incubation: 14 days	28 days	38 days	38 days
	Dry weight of mycelium (g.)			Vacuum (in.)
	Small headspace			
15	Trace	0·008	0·004	12·1
20	0·022	0·017	0·019	9·1
25	0·021	0·020	0·021	9·2
32	0·025	0·018	0·027	7·5
38	0·035	0·018	0·032	5·3
	Large headspace			
15	0·002	0·039	0·043	9·9
20	0·052	0·033	0·031	8·6
25	0·067	0·042	0·053	6·8
32	0·052	0·037	0·042	6·7
38	0·043	0·046	0·046	5·2

It was concluded that with a large headspace maximum growth took place at 20–38° C. within 14 days, but within 28 days at 15° C. With a small headspace maximum growth was lower than with a large headspace. It was attained within 14 days between 20 and 38° C. but growth remained slight at 15° C. even after 38 days. Thus if, in practice, the cans are stacked while still warm and heat diffusion from the stack is slow, the development of mycelium will be greater than if the cans are cooled thoroughly before stacking.

V. Summary

1. A survey of various fruit-producing areas in England showed that fruit and foliage were liable to be contaminated with ascospores of *Byssochlamys fulva*. Contamination was most pronounced on mummified plums and on certain fruit refuse.

2. The thermal death point of ascospores is about 96° C., but only a small proportion of spores show this maximum heat resistance. Conidia are killed by exposure to relatively low temperatures.

3. The reaction and the sucrose concentration of the heating medium, and the strain of the fungus, are factors influencing the heat resistance of ascospores.

4. Ascospores germinate slowly and irregularly, but their germination is stimulated by previous heating to about 70° C. Ascospore germination and mycelial growth take place at relatively high temperatures, in high sucrose concentrations, in acid media, and in very high concentrations of carbon dioxide.

5. Ascospores are relatively insensitive to antiseptics and to desiccation.

6. Growth is accompanied by the production of a pectin-destroying enzyme.

7. The results of laboratory experiments were applied to canning experiments and good agreement was obtained. Natural contamination on fruits could be overcome in cans by heating the contents to 195° F.

8. The amount of mycelial growth developing in an infected can depends upon the size of headspace and the storage temperature.

The writer wishes to thank Prof. V. H. Blackman and Prof. W. Brown, under whose direction this work was done; also the Director and Staff of the Campden Research Station for help in planning and executing the canning experiments. Thanks are also due to the Agricultural Research Council for a grant towards the cost of the investigation.

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NOTE ON THE LIMITATION OF INFECTION OF WHEAT BY ASCOSPORES OF *OPHIOBOLUS GRAMINIS* SAAC. A POSSIBLE EXPLANATION

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Introduction

Garrett (1939) demonstrated that failure of infection of wheat by ascospores of *Ophiobolus graminis* Sacc. may be due to antagonism by other organisms, and that this may be intensified by a low nutritional level for the invading hyphae. He states that "In wheat seedlings grown under pure culture conditions in sterile sand, the excretion of organic material from the growing roots may provide the accessory nutrient necessary for successful ascospore infection" and adds that "in unsterilized soil, the root excretions are readily assimilated by the general soil microflora".

In view of Garrett's experiments and the interpretation put upon them, the results of an experiment conducted in 1933 at the Imperial College of Science and Technology, London, are of interest. It was conducted in order to test whether the fungus is homothallic, as suggested by Davis (1925).

Experimental

In October 1933 Dr W. A. Millard of Leeds University kindly provided ascospore material of *O. graminis*. A dilute spore suspension was streaked on Petri dishes of potato dextrose agar, and a number of single spores, well isolated from any others, were transferred by means of a finely pointed dry wire needle to sloped tubes of potato dextrose agar. Forty-five spores were treated in this way and the tubes were kept in a dark cupboard. The day following removal of the spores, abundant germination of those remaining in the dishes was seen.

The tubes were observed daily for a fortnight, and by the fifteenth day colonies had not appeared. On removing the tubes a week later a minute colony was found in one tube and proved to be *O. graminis*. Each day thereafter for nearly a week one or two more tubes showed minute colonies, which afterwards grew rapidly. In all, 14 spores germinated by the end of 1 month, after which germination ceased.

Each of the 14 single spore colonies was mutiplied in a flask of a sterilized mixture of 90 g. of sandy loam soil, 10 g. of finely ground maize-meal and 50 c.c. of water. After 6 weeks, when abundant growth had occurred, the contents of each flask was used to infest a pot of sandy loam soil previously autoclaved at 15 lb. pressure for 1½ hr. Twenty-five seeds of Little Joss wheat were sown in each pot on 5 January 1934. By 26 February all the wheat plants inoculated with the various single spore cultures of *Ophibolus* were stunted and killed by the fungus, whereas control plants were healthy. Black incrustations of perithecia were found at the bases of plants in 12 of the 14 different inoculated series. In 9 of the 12 the ascospores were mature and

readily germinated. There was thus no doubt that this fungus is homothallic, as demonstrated by Davis (1925).

Discussion

The behaviour of the spores when isolated was interesting. The fact that excellent germination was seen in the remaining spores on the Petri dishes following the removal of the single spores had led to the conclusion that the transferred spores must have been injured mechanically with the needle, an explanation disproved when they germinated. Apparently the only satisfactory explanation for hindrance of germination was that some substance produced by the ascospores themselves or by the perithecia, required for the germination of the spores, became so diluted on their removal to fresh agar as to result in long delay in germination.

Since ascospores of this fungus are produced abundantly under the conditions prevailing in certain countries, the question of spore germination is of practical importance in relation to dissemination of the fungus. If the results reported above can be repeated it means that ascospore infection under natural conditions is extremely unlikely, because during the period when susceptible plants are available it will be impossible to get sufficiently abundant concentration of spores for rapid germination. If a growth substance is required for spore germination, it is probably different from that which is required for hyphal growth (Padwick, 1936), since that substance is present in abundance in potato dextrose agar, on which the single spores germinated so slowly.

In his inoculations, Garrett used a concentration of 80,000 spores/c.c. This concentration is much greater than normally occurs in nature, but even so it is interesting to calculate the degree of concentration of spores. The volume of an ascospore, assuming it to be a perfect cylinder of size $100 \times 3{\cdot}5\mu$, which is somewhat larger than the figures given by Kirby (1925), is $\pi r^2 l = 3{\cdot}1416 \times (1{\cdot}75)^2 \times 100 = 962$ cu. μ. One c.c. contains $(10{,}000)^3$ cu. μ, so that for each of the 80,000 spores there will be

$$\frac{1}{80{,}000} \times (10{,}000)^3 \ (= 12{,}500{,}000) \text{ cu. } \mu$$

of solution, and the ratio of suspending liquid to spore volume will be

$$12{,}500{,}000 : 962 = \text{approximately } 13{,}000 : 1.$$

The degree of dilution of any soluble substance produced with or by the spores is thus very great.

What may probably have happened in Garrett's experiments is that the spores germinated very slowly owing to their dilution. This slow germination did not matter in the case of sterile conditions, since eventually the spores could utilize whatever growth substance was available and eventually cause infection. The delay was, however, fatal when the spores were faced with competition for nutrients by other fungi, or when the unfavourable nutritional conditions were enhanced by toxic substances produced by these or other organisms.

This interpretation may be disproved, but these results obtained 6 years ago may prove an interesting point to workers on *Ophiobolus*. In 1936 I stated "what has been demonstrated is...the importance of studying the nutritional aspect when considering questions of antagonism and growth of the fungus in soil". By a different method of approach, Garrett and I have reached the same conclusion. He says "It

is suggested, therefore, that microbiological interference with the initiation of ascospore infection is a competitive rather than an antagonistic effect, and is due to assimilation by the general soil microflora of the nutritive substances excreted from the growing and developing roots." I stated that "It is as reasonable to suppose that *O. graminis* fails to grow in unsterilized soil, at least in some cases, through lack of growth factor, or through competition for growth factor by other organisms, as it is to regard the control exercised as being entirely due to production of some inhibitory substance by these organisms." It may be that ascospores and hyphae differ mainly, physiologically speaking, in mass, or, what amounts to the same thing, "nutritional level". At any rate, we seem to have approached much nearer to the truth than we had a few years ago before it was demonstrated that *O. graminis* is a fungus of rather unusual nutritional requirements.

Summary

1. Single spore cultures of *O. graminis* Sacc. produced ascospores on the host, confirming the conclusion of Davis (1925) that the fungus is homothallic.

2. Single spores removed from a Petri dish took several weeks to germinate, whereas the spores remaining in the dishes germinated within 24 hr.

3. It is suggested that a retardation of spore germination may have been due to dilution of some necessary chemical substance.

4. The significance of slow spore germination in relation to the results obtained by Garrett (1939) on infection of wheat seedlings by the ascospores of *O. graminis* is discussed.

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PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Annual Summer Meeting held in the University of Manchester, 8–10 June 1939. A paper reading session was convened in the Natural History Theatre, Zoology Department, on Friday, 9 June, at 10 a.m. The Chair was taken by the President, Mr C. T. Gimingham.

Symposium on Applied Biology and Crop Production

The following papers were read:

I. Pedology, the study of the soil. By Prof. G. W. Robinson, M.A.
II. Botany and ecology in relation to crop production. By G. H. Bates, D.Sc.
III. Agricultural entomology in Yorkshire. By H. W. Thompson, M.Sc.
IV. Some past and present crop disease problems in the north of England. By F. T. Bennett, Ph.D., D.Sc.

I. PEDOLOGY, THE STUDY OF THE SOIL

By Prof. G. W. Robinson, M.A.

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I assume that, as Applied Biologists, your interest in the soil is principally in its function as a medium for plant growth, and that you are acutely aware of the magnitude of the problems that centre in the relationships between the soil and the natural vegetation or cultivated crops that grow on it. My own special interests lie more particularly in the study of soil as a natural body, but I make no apology for this, because I believe that the problems of plant growth can be most successfully solved through a clear understanding of the nature of the soil medium, and an equally clear understanding of the mechanism of plant nutrition in soils. Whilst I recognize the immediate practical value of investigations in which the effect, measured in crop yield, of any given soil treatment is studied, it seems to me that the results of such work can be, with certain exceptions, of only limited significance and application.

During the past generation there has been a fundamental change in the methods and ideals of soil investigation. Whilst the soil was formerly regarded as a material in which crops grow, in the modern study of the soil it is regarded as a natural individual to be studied as such by the methods of pure science.

The great advance in the study of soils during recent years has been in the recognition of the importance of soil morphology. In the modern view, soil is not simply

a material but an individual with differentiation of parts. The soil individual recognized in modern soil studies is the soil profile, which consists of the succession of horizons occurring in vertical section from the surface down to the geological material from which they have been differentiated by the soil-forming processes.

Study of soil as a material, although inadequate to characterize a soil, is nevertheless necessary, if only to furnish a basis for the comparison of the horizons that make up the soil profile. It is, therefore, necessary for us to consider briefly the constitution of soil material in the light of modern knowledge.

Soil is developed from the products of weathering of the rocks of which the earth's crust is composed. In it we may recognize fragments of rock and of rock-forming minerals. The mineral portion of the soil is, in the main, the product of geological weathering and differs from it principally in being associated with the more or less altered products of decomposition of plant residues, known as *humus*. It differs also from rock débris in being the seat of a micro-flora and fauna, and in possessing a definite structure. Viewed in another way, we can distinguish in the material of soil: (*a*) unweathered rock and mineral fragments and undecomposed plant residues retaining their original structure, and (*b*) secondary products formed from the chemical weathering of silicate minerals and from the microbiological decomposition of plant residues. The secondary material formed by the chemical weathering of mineral silicates forms the *clay complex*, whilst the material formed from the microbiological decomposition of plant residues forms the humus of the soil. The clay complex is the result of processes that are to some extent geological. Indeed, in soils derived from deposits, such as Gault Clay, that have been only slightly metamorphosed, the clay complex is the product of an earlier weathering cycle. Humus, on the other hand, is the product of contemporary microbiological processes within the soil. Clay and humus occur in intimate association and together form the so-called *colloidal complex* of the soil. The colloidal complex is the reactive part of the soil, and occurs partly as independent aggregates, partly as films or coatings investing the less reactive skeletal material and causing its aggregation into compound particles or crumbs.

In the mechanical analysis of soil, the particles of which the soil is composed, after preliminary treatment to break down compound aggregates, are separated into different grades or fractions. The relative proportions of these fractions determine the texture of the soil. Broadly speaking, we may say that particles greater than about 2 mm. diameter consist of rock fragments, particles between 2 and 0·002 mm. are mainly fragments of unweathered minerals, such as quartz, felspar, micas, and ferromagnesian minerals, whilst the material less than 0·002 mm. diameter is mainly the clay complex.

The clay complex is, as we have seen, secondary material formed from the weathering of silicate minerals. It appears to consist of certain platy crystalline minerals, which may be described as hydrated aluminoferric silicates, sometimes associated with hydrated sesquioxides and, possibly, complex colloidal gels of indefinite composition.

The principal constituent of humus is a complex formed by the condensation of proteins or their hydrolytic products with altered lignins. It is relatively resistant to microbial attack, and must be considered in the main as chemically inert. Most of the unavailable nitrogen of soils must be assigned to the category of humic nitrogen.

The colloidal complex, considered as a whole, behaves as a weak insoluble acid.

In ordinary soils, this acidity is more or less neutralized by bases, the so-called *exchangeable bases*, of which the principal is calcium. In equilibrium with excess calcium carbonate a soil is *base-saturated*. On the other hand, in very acid soils, comparatively small amounts of exchangeable bases are present. In the complete absence of exchangeable bases the soil is said to be *base-desaturated*, a condition closely approached by many of our mountain soils in wet regions. The base-status of soil is reflected in the reaction or *p*H of the soil moisture. At complete base-desaturation the *p*H is approximately 4·0. Lower *p*H values are generally due to the presence of small amounts of sulphuric acid formed from the microbiological oxidation of organic sulphur compounds.

The prevalence of very acid reactions in the soil markedly affects microbiological decompositions. Bacterial decomposition becomes recessive to fungal decomposition and plant residues tend to accumulate at the surface as peaty raw humus, a condition that is accentuated by the more or less complete absence of earthworms. Under the same conditions, and owing to the presence of acid humus, the clay complex undergoes a decomposition whereby ferric oxide and, to a lesser degree, alumina, become mobile and are washed down from the surface horizons to be deposited at lower levels in the profile. This is indicated by a bleaching of the soil immediately below the raw humus layer and the development of rusty or yellowish colours below.

We are now in a position to consider the origin and development of soils as individuals. At this stage, it will be convenient to distinguish between rock weathering and soil formation. The distinction can be most clearly appreciated in the tropics, where the weathering of rocks has proceeded much more intensively and for a longer period than in our climate. It is not uncommon in humid tropical climates to find 50 ft. or more of weathered material overlying the rock from which it has been formed. In such cases it is plain that the soil has been developed from the weathered material by a distinct group of processes associated with, and conditioned by, the presence of a vegetative cover. The soil-forming processes are equally distinct in those cases where the parent material consists of unmetamorphosed or lightly metamorphosed sediments resulting from an earlier cycle of weathering and decomposition. In a climate such as our own, the zones of soil formation and rock weathering generally overlap. In fact, some soil profiles are so shallow that the soil profile extends down into the rock itself.

The weathering of rocks belongs more appropriately to the sphere of geology. We may note, however, two types of weathering, namely physical and chemical. In physical weathering, the changes involved are merely mechanical comminution, and are the result of such agencies as temperature changes, frost and thaw, moving water, or moving ice. In chemical weathering, which may be concomitant or subsequent to physical weathering, chemical decompositions take place, whereby certain constituents are lost and secondary products formed. We shall make no serious error in equating the clay complex of soils and clays with the secondary material formed as a result of chemical weathering.

The development of soil from weathered geological parent material is conditioned by the presence of a cover of vegetation. The dead remains of plants, consisting mainly of fibrous materials, undergo microbiological decomposition in the soil and build up a content of humus. Plant residues added to the soil from the death of roots are at once incorporated with the soil. The residues of the aerial parts of plants become

incorporated with the soil mainly through the agency of earthworms, except in the case of very acid soils, where they accumulate as a raw humus mat.

The character of the superficial accumulations of humified and semi-humified plant residues or litter, the vertical changes in humus content within the soil profile itself, and the sharpness of these changes as judged by appearance in vertical section, are of the highest importance for diagnostic purposes.

There are, however, other processes in profile development, affecting the mineral constituents of the soil. These are governed by the movements of water in the profile. In a humid climate, such as our own, there is an excess of rainfall over evaporation, and this excess is disposed of by downward percolation to the drainage. With impervious subsoils, or in the presence of high water-tables, the downward movement is limited to the zone of free percolation, and is then lateral in direction.

Material removed in solution from the upper horizons of the soil may be lost completely from the soil profile and pass into the drainage. This is the case with soluble salts of the alkalies and alkaline earths. In other cases, and particularly with material in suspension or colloidal solution, deposition may take place in a lower horizon. There is thus a differentiation into a superficial horizon, termed the *eluviated* or *A-horizon*, and a lower horizon termed the *illuvial* or *B-horizon*. The character of the eluviation differs in different classes of soil profile. The simplest case is where eluviation is purely mechanical, involving the translocation of fine material, and resulting in differentiation into a coarse-textured A-horizon and a fine-textured, often compact, B-horizon.

Where eluviation takes place in the presence of acid humus, as under coniferous or heath vegetation, the colloidal complex becomes unstable and sesquioxides, principally ferric oxide, are removed from the A and deposited in the B horizon. This is shown by a bleaching of the superficial layers and the development of orange or rust-brown colours in the B-horizon. In extreme cases, humus may be deposited at the top of the B-horizon. The fully developed profile in such circumstances is the well-known *podsol profile*. As this, or one of its variants, is the most characteristic profile under natural vegetation in northern England, Wales and Scotland, it may be useful to give a description of a typical example, such as may be found under heath vegetation or under long-established coniferous forest.

Beginning from the surface, the first horizon consists of litter in the first stages of alteration. Below this is more or less humified litter, which, however, still retains something of its original structure. These horizons together form the A_0 *horizon*. They are succeeded by the A_1 *horizon*. This varies in thickness from a mere rudiment to a few inches, and consists of the mineral soil mixed with dark structureless humus. Below it is the A_2 *horizon*, which has a bleached or grey appearance and, when dry, a loose or, occasionally, laminated structure. The thickness is variable, and rarely amounts to a foot. Bleached sand grains are characteristic of the A_1 and A_2 horizons. Below the A_2 horizon there succeeds, with sharp transition, the B_1 *horizon*, which is dark-coloured or even black owing to deposition of humus. In this horizon there is a marked increase in degree of compaction. The thickness varies from a fraction of an inch to 2 or 3 in. In some profiles, including most Welsh profiles, the B_1 horizon is absent or indistinguishable. Below the B_1 horizon is the B_2 *horizon*, characterized by yellow, orange, or rust-brown colours owing to deposition of hydrated iron oxides. The B_2 horizon varies in thickness from a few inches to 2 ft. or more, and merges

with varying distinctness into the *C-horizon*, which is the parent geological material.

Most of the well-drained soils of the humid north and west of Britain are variants of the podsol profile. A profile such as the one described may be termed a developed or mature profile. Where the natural profile has been changed, owing to cultivation, we have agricultural profiles, in which may be distinguished grassland and arable profiles, respectively. It is obvious that when a virgin soil is cultivated, all horizons down to the depth of tillage are mixed together and lose their identity.

Following on cultivation, erosion may remove some of the surface horizons, thus giving truncated profiles. Many of our Welsh upland soils are of this class, the present top soil representing the former B-horizon. Apart from modifications induced by cultivation and erosion, there are variations in the degree of maturity. Profiles in the earlier stages of development may be termed *immature*. Even given the same conditions of development, there are also variations depending on the character of the parent geological material. Podsolization is favoured by a low mineral status in the parent material. Consequently, development will be more pronounced on highly siliceous sands with low base content than on materials such as shales, which, although not calcareous, may contain reserves of weatherable minerals. Where parent materials are calcareous, podsolization cannot occur so long as calcium carbonate is present in the soil horizons.

The problem of soil classification has occupied considerable attention for many years, and it cannot yet be regarded as satisfactorily solved. Perhaps the principal difficulty has been the incompleteness of our knowledge of the soils of the world. A system of classification that is adequate for the soils of a region or country is generally found to be inadequate when applied to other regions. Since it is only during comparatively recent years that methods of characterizing soils as individuals have been developed, the materials for a system of classification are still incomplete. Nevertheless, certain world groups have been recognized and the broad lines of a classification may be given.

We may distinguish in the first instance three great classes or groups. First, there are *soils with completely leached profiles*. These are soils with free drainage in humid climates. Secondly, there are *soils with incomplete leaching* owing to the predominance of evaporation over rainfall. There are the soils of arid climates. Thirdly, there are *soils with impeded or restricted leaching*, occurring both in humid and arid climates.

The first group may be subdivided according to the presence or absence of an acid humic surface layer. In the presence of such a layer, we have the *podsols* and their congeners. Where an acid humic layer is absent, we have the *brown earths* or *brown forest soils* of temperate climates and the *red loams* and *lateritic soils* of the humid tropics. Such soils, although acid and free of carbonates, do not show bleached A-horizons and sesquioxide accumulation in the B-horizons.

The second great group may be subdivided according to differing degrees of leaching, varying from the *tshernosems* or *black earths* to the *desert soils*, the presence of an horizon of calcium carbonate accumulation being a constant feature.

The third great group can be at once subdivided according to the presence or absence of sodium salts. In the former case we have the saline and alkaline soils; in the second case we have a range of soils varying from so-called *gley soils*, characterized

by grey colours and rusty mottlings or patches in the region of the water-table, to the *peats*.

The categories distinguished by this primary classification may be further subdivided according to the stage of development of the soil profile, recognizing such variants as *eroded* and *agricultural profiles*. Finally, distinctions may be drawn on the basis of parent material and texture.

It will be realized that the number of possible varieties of soil is very large. The problem of classification is rendered still more complicated by the occurrence of transition types. The final categories have not the definiteness of genera and species in plants and animals, but often represent ideal stages in a continuous succession, analogous to the ideal granites, syenites, and gabbros of the petrologist.

I feel that I should be avoiding an important aspect of pedology if I omitted some discussion of the mutual relationships of soil and vegetation. The problem presents itself to you as applied biologists in such questions as these. What information about the soil should be collected in order to specify soil conditions in ecological studies? What characters of the soil are critical for plant growth? What soil characters determine the occurrence and distribution of ecological units?

Before attempting to arrange the different soil characters in order of importance, I will briefly enumerate the headings under which information is desirable in describing soils from the point of view of their effect on plant growth.

In the first place, climatic and topographical data must be recorded, with special attention to micro-relief. With these data may be included information on the hydrological conditions and a general statement of the superficial geology. I assume that biologists will collect all data on vegetation.

The next step is to describe the actual soil profile. This can only be examined in a section, best obtained by digging out a hole or trench. The depth necessary to include the complete profile varies, but would rarely exceed about 4 ft., to include all the pedological horizons. It is generally advisable to secure a natural face to the vertical section by breaking off the surface. Examination of a face smoothed or compressed by digging implements may give misleading results. Inspection will now enable the observer to recognize the different horizons down to the parent material. These horizons should now be described in succession from the top. For each horizon, data should be recorded concerning thickness, colour, texture, stoniness, organic matter, structure, and moisture conditions. If possible, the reaction should be determined on the *p*H scale, and the occurrence of carbonates noted, if present. Any secondary depositions such as rust marks in root channels, and concretions or deposits of iron oxides or manganese dioxide should be recorded.

Certain laboratory data are also desirable, and samples for analysis should be taken from each horizon. It should not generally be necessary to carry out complete analyses, but it would be desirable to determine the organic carbon, the *p*H, and, if possible, the mechanical analysis. The organic carbon multiplied by the conventional factor 1·724 gives a measure of the organic matter. Loss on ignition as a measure of organic matter is entirely untrustworthy.

In conclusion, I will attempt to indicate the soil characters which appear to me of most importance for plant growth and which would appear to determine the occurrence and distribution of different vegetational units, so far as these are determined by soil conditions.

So far as the growth of natural vegetation is concerned, it would appear that two soil factors are of outstanding importance, namely, (1) the air-water regime within the soil, and (2) the base-status.

I group aeration and moisture together because excess of the one implies defect of the other. In the last analysis, practically all limitations imposed by the soil on the growth of plants operate through the air-moisture regime. Thus, shallowness implies liability to drought through restriction of root development. Coarse texture implies low moisture-holding capacity and excessive oxidation of organic matter. Heavy texture implies deficient aeration and restricted root development during the wetter months and liability to drought during the summer.

The importance of the air-moisture regime is evident when agricultural soils are considered. Human interference can effect much by modifying unfavourable soil conditions and building up fertility. It is safe to say, however, that our most fertile agricultural soils are those in which the factors water-supply and air-supply operate most favourably. Such soils are found by experience to repay good cultivation and manuring and therefore acquire good plant food status.

The other factor that appears to be of critical significance is the base-status of the soil. It is possible to view this property of the soil from more than one standpoint. Most usually the base-status of the soils is visualized in terms of the reaction of the soil moisture as expressed by the *p*H value. It is true that the *p*H does reflect the acid-base regime of the soil. It is equally true that excessively acid and excessively alkaline reactions may directly exert an adverse effect on plant growth, and that the critical reaction may vary for different plants. One must not, however, accept the significance of *p*H too uncritically. For one reason, the accurate determination of the *p*H of the soil is a matter of considerable difficulty. Apart from this, however, it must be noted that the *p*H of the soil is an expression of a property which is governed by a number of factors, principally by the nature and amount of the colloidal material present, and by the nature and amount of the associated exchangeable bases. It may be, therefore, that the critical factor for plant growth is not actually the *p*H, but the base-supply. From my own experience of agricultural soils in North Wales, I am disposed to attach greater importance to the actual amount of lime in the soil than to the reaction as measured by *p*H. And so, whilst *p*H data may give useful indications, they should be used with great caution in ecological studies.

Equal caution is needed in the use of oxidation-reduction potential measurements. Whatever may be its significance, the determination of oxidation-reduction potential is beset with enormous difficulties. In fact, some workers have even come to the conclusion that oxidation-reduction potentials only represent inaccurate *p*H determinations.

In the present state of our knowledge of the soil, we are still far from being able to effect a complete correlation between soil profile characters and plant growth. This is due as much to imperfections in our knowledge of plant nutrition as to the inadequacy of our pedological knowledge. The principal problem of the pedologist is to understand, describe, and classify his material. In this way he can most efficiently contribute to the solution of the problems of the growth and distribution of plants.

(*Synopsis*)

II. BOTANY AND ECOLOGY IN RELATION TO CROP PRODUCTION

By G. H. Bates, D.Sc.

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In reviewing so wide a field it is not possible to deal with every aspect of the subject in detail. Probably the most satisfactory procedure is to consider those contributions which, in the writer's opinion, are the most important from the cultivators' point of view.

In plant breeding the application of Mendelism has been a great advance on the methods of the past, it is more economical and more productive of results. On the other hand we appear to have gone as far as Mendelian methods can take us, and have arrived at a point where we have rung the changes on all the available material.

It is probably for the above reasons that such a wave of optimism has swept through the ranks of geneticists after the advent of the colchicine method for the production of polyploidy. It is possible to produce fresh material to work upon, but whether results up to date can be regarded as being more than encouraging is a matter of doubt.

There has been a revival of the study of graft hybrids and attempts have been made to produce disease immune potatoes by building up periclinal chimaeras with an "outer skin" of a disease resistant variety. We have also learned to understand the constitution of some of our existing varieties.

Greater attention is being given to root studies. For obvious reasons our knowledge of underground organs lags far behind that of aerial parts. New technique is allowing for observations on the development and reactions of roots. This has been made possible by the introduction of glass chambers and shafts. It is possible that the plant breeder may be able to make use of root characters in producing strains resistant to drought, disease and lodging, once he has been enabled to identify those characters.

Work on growth promoting substances has cast light on growth mechanism flowering and tuber formation. Results as a whole are conflicting and contradictory, as may be expected in a new field. Some concrete achievements have emerged and synthetic products are available for the stimulation of root development.

Investigations on dormancy and methods for breaking the resting period have not received the prominence they deserve. Apart from the well-known treatment of bulbs, there appear to be distinct possibilities for the application of chemical treatments for breaking dormancy to potatoes.

The new technique for plant injections has opened a wide field of investigation. There is a better understanding of the vascular system and movement of water, owing to the fact that certain dyes may be taken into the plant without causing injury. It is hoped that the system may be used for diagnostic and curative purposes on a greater scale than at present. The results of investigations carried out in this field cast valuable light on problems which have arisen in connexion with a new method of bracken destruction by the application of sodium chlorate to the cut surfaces of the stems.

In the sphere of ecology there is need for a great enlargement of the work done on

the aut-ecology of certain weed species. A good deal of evidence has been produced pointing to the relationship between weed flora and cultivations.

In grassland improvement it might be said that the whole of the work carried out at Aberystwyth is really applied ecology. The production of indigenous strains and types suited to the particular habitat has been a great step forward. Methods of improvement of hill land show an appreciation of the concept of sequence and climax.

(*Synopsis*)

III. AGRICULTURAL ENTOMOLOGY IN YORKSHIRE

By H. W. Thompson, M.Sc.

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Entomological problems of one Advisory Province must, generally speaking, bear a close resemblance to those of other corresponding areas. Consequently, a brief outline of the type of advisory work met with in Yorkshire will, it is hoped, give an indication of the general scope of agricultural entomology and its relation to crop production.

Yorkshire is large and varied, practically every geological formation occurring in the county, with the result that practically every type of soil exists and, therefore, every type of farming. Physically the county is made up of four upland masses with lowland areas between and about them. To the west of the county lie the Pennines, almost continuous from north to south but broken by the Aire Gap which allows the passage of river, canal, roads and railway. In the east of the county we have the North Yorkshire Moors which are largely heather covered, and farther south, in the East Riding the Wolds, which 100 years ago were mainly rough sheep grazing land but are now 75% arable and form an important cereal-growing area.

All the central part of the county, and roughly half of it, is taken up by the Plain of York, which extends from north to south of the county. Running eastwards from the plain there are in the north, the Vale of Cleveland, in the centre the Vale of Pickering and in the south on Humber side the Holderness area which includes the land adjoining the tidal rivers, Ouse and Derwent, where extensive warping has been carried out, mainly 30–60 years ago, but some of it in recent times.

Main crops and pests of the areas indicated

(1) *North Pennine region*

This area which is largely mountain limestone, with some millstone grit, is an important grazing area, and in the main has no big entomological problem. Pests which occur at intervals are leatherjackets and garden chafers causing turf injury, antler moth and on one occasion fescue aphis causing severe herbage injury, and the heather beetle on grouse moors, which causes widespread injury.

(2) *Southern Pennine region*

This area includes the industrial south-west region of the county and lies on millstone grit and coal measures' formations. It has large areas of allotments and extensive market gardens on which all the common vegetable pests occur and are

troublesome at times. Important special crops which are grown intensively locally are broccoli and rhubarb. On the former, cabbage-root fly attacks are often serious, although now checked to some extent by more general use of deterrents. Rhubarb has few pests, but one, the stem eelworm (*Anguillulina dipsaci*), which has now been shown to cause a crown-rotting condition, is a serious local problem. As this eelworm has a very big host list, the attack on rhubarb introduces complications in the selection of a rotation on land on which rhubarb is to be grown for forcing.

Peas for picking green are largely grown on the fringe of the industrial area, and on this crop heavy attacks of the pea thrips frequently occur. Early peas are often followed by wheat, and it is found that attacks of wheat bulb fly are liable to follow the partial fallowing after the early ploughing of the peas. Such attacks are liable to be severe, as much of the land in this area is too light to be ideal for wheat.

North Yorkshire moors.

In this part of the county conditions and pests are similar to the north Pennine region and there is little as a rule of entomological interest. At one centre, however, watercress is grown on a large scale, and mustard beetle attacks have proved a very serious problem in the past. *Derris* could not be used owing to adjoining fish hatcheries, but satisfactory control has been obtained by pyrethrum spraying.

East Riding wolds.

This interesting region is an important barley-growing area, and on this crop gout fly attacks occur from time to time, but usually appear to be more severe on the lower ground around the wolds rather than on the top.

It is common practice here to plough out clover in late autumn and sow wheat at once, and severe frit fly attacks on winter wheat often result from the migration of the larvae from the grasses in the leys to the wheat. Summer ploughing of leys which would prevent such an attack is not practicable for various reasons on the wolds.

In 1938 attack of the wheat shoot beetle (*Helophorus nubilus*) were severe on crops which owing to an unfavourable seed time and winter were very backward. This pest was recorded as early as 1920, but is not usually severe. Attacks follow the ploughing out of clover, which is commonly sown alone for sheep grazing.

Swedes complete the cropping of the wolds. Owing to the low rainfall of this area and its soil characteristics, early growth of this crop is often very slow and in consequence attacks of turnip flea beetle are frequent and severe. Seed dressings to prevent attack are often used, not always successfully. The open chalky soil favours earwigs, which are very prevalent and often cause injury to the swede crop.

The Plain of York

This region is, generally speaking, very fertile and here farming reaches a high standard. All farm crops are grown, the most serious pest being the frit fly. Winter oats are not grown to any great extent. In some years, when weather conditions make it necessary to sow spring oats late, or when the young plants are checked in growth, the danger of severe attack is great.

In the northern part of the plain, oats are grown on a very large scale, often at short intervals and stem eelworm attacks frequently occur. During the last few years the cereal strain of the root eelworm (*H. schachtii*) has been found to be prevalent also.

Sugar beet is grown to the extent of about 30,000 acres in the centre of the county. In the last few years the serious beet pests have been cutworms, mangold fly, and black aphis. No case of "beet sickness" has been met with so far in the county and, with present restrictions on the too frequent growing of beet, should never develop into a serious problem in the county.

Large acreages of carrots are grown south and east of York, and carrot fly causes heavy loss in some years. Deterrents are now being tried on a field scale by some growers, but the general opinion appears to be that over a period of years losses would not be sufficiently heavy to justify annual applications of insecticides.

The southern part is the main potato-growing area, and extends to the warp areas around the Ouse and Derwent. This latter area is very suitable for wheat as well as potatoes, and on many farms these two crops were grown in alternate years in the past with the result that much of the land developed "potato sickness". The need for longer rotations is, however, generally recognized now, so that potato crop failures due to eelworm have become rare on a farm scale.

Many farmers in this area submit soil samples each year for cyst examination before arranging their potato acreage. The remarkable power of recovery of wheat grown in this district overcomes to a large extent danger of loss from wheat bulb fly, which is prevalent.

Large acreages of field beans are also grown on the Humber side and usually do extremely well, although in some years stem eelworm (*A. dipsaci*) causes heavy loss to this crop.

Fruit

Good fruit can be grown in the county; there are some 3000 acres of orchards, but very few fruit growers on a large scale. Generally speaking fruit culture is rather backward, few growers have a regular spraying programme, and many do little or no spraying. Consequently heavy aphis attacks are common. Other common pests are winter moth, capsid bug in the southern half of the county, codling moth and apple sawfly, the former having become more prevalent during the last few years. Apple blossom weevil has been found at two centres only, and must have been absent or nearly so until the last year or two.

Attacks of magpie moth and gooseberry sawfly on bush fruit are of general occurrence.

Strawberries are grown commercially on a large scale near Hull, and on this crop aphides, red spider, and strawberry eelworm are the main pests.

Glasshouse crops

Glasshouse cultivation has developed enormously in the last few years, particularly in the amount of Dutch houses with removable lights. Tomatoes, cucumbers and chrysanthemums are grown extensively, and all the common pests of these crops are present. In addition we have one special problem in Yorkshire which is the attack of the root eelworm (*H. schachtii*) on tomatoes causing "tomato sickness". This attack was first noticed in 1928, and we now have records of attack at almost 200 centres. An account of this attack has been published (Johnson & Thompson, 1936) and need not be re-stated; since it was written further observations have been made on the effect of change of soil, of steam sterilization on the cyst content of the soil, and on the effect of chemical dressings applied to infested soil.

Control

(1) A good crop can be obtained by changing the soil to a depth of 15 in. This may allow two tomato crops provided the subsoil infestation is not heavy, and is useful in small houses and those awkward to steam.

(2) Steam sterilization when done thoroughly is effective and should allow two satisfactory crops to be taken, but not more, and some growers favour a rather lighter steaming each year.

(3) Dutch houses may be moved to eelworm-free sites; this can be done at a less cost than steam sterilization and in the absence of eelworm such houses are usually kept on one site for 10 years.

(4) In houses where steaming is not possible, nor regular change of soil, some safeguard can be obtained by watering the beds with corrosive sublimate solution at 1 in 250 strength, using 1 gal./sq. yd. 3 weeks before planting.

Attacked plants can be helped to some extent by paying particular care to cultural details or by layering into or mulching with eelworm-free soil to promote surface rooting.

In Yorkshire the areas where "tomato sickness" occur coincide with the main potato growing part of the county, where "potato sickness" is also prevalent. Cases have come to notice where both Dutch and permanent houses have been erected on "potato sick" land, and "tomato sickness" has developed on the first year's crop. The mature tomato strain cysts appear indistinguishable from the potato strain cysts; they fall within the same size range and are believed to be identical.

For general purposes in giving advice, the presence of 10 viable cysts per 20 g. of dried soil is regarded as the danger mark, and this figure has worked out well in practice.

With an infestation below that figure a satisfactory crop may be obtained; if far above it, severe sickness is likely to occur.

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IV. SOME PAST AND PRESENT CROP DISEASE PROBLEMS IN THE NORTH OF ENGLAND

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The mycological problems in the four northern counties do not differ in kind from those in other provinces, but they are fewer in number and of comparatively greater local economic importance. This arises from the fact that the kinds of farm crops and horticultural activities are restricted by geographical and climatic influences. The geographical features are the hill masses of the Cheviots and Northern Pennines, somewhat T-shaped, with the Cumbrian mountains occupying the greater part of the space in the left arm. In these uplands sheep farming is practised, there is little arable

land, rarely as much as 5% of the farm being tilled, and the small arable crops are consumed at home. In the more extensive arable area, the eastern slopes of the Pennines to the North Sea, climatic conditions have a restrictive influence. The mean average temperature is low; at the Northumberland County Farm (Cockle Park), typical for the area, the mean maximum is 52·5° F., and the mean minimum 39·5° F. Night frosts are common in May, and at times occur to the end of June. In spring and early summer there are "sea frets"—low cloud conditions, damp and sunless, which retard the drying and warming of the soil. Under these conditions a large proportion of land is under permanent pasture or long leys which provide no mycological problems; the arable crops are of comparatively few kinds, horticulture is limited, and fruit growing on a commercial scale is absent. As the kinds of crops are relatively fewer the mycological problems concerning them assume comparatively greater importance, and call for special attention in relation to the local climatic conditions and farm practices. Of the farm crops the problems are concerned with swedes, cereals and, to some extent, potatoes, and the following notes indicate past, present and probable future developments.

Club root (Plasmodiophora Brassicae) was formerly the limiting factor in root husbandry. Control of this disease by lime, first demonstrated at Cockle Park, is now the standard practice everywhere. Frequently in the north, however, it is impossible to apply economically the amount of lime required for good control of club root on these notoriously sour soils. The problem was investigated, therefore, as to the use of resistant varieties, and the use of soil dressings which would assist this insufficient liming. As regards the latter, no substance has yet proved successful and economical under field conditions. As regards the former, resistant varieties were tested for several years, and finally nine most promising ones were tested extensively under two sets of conditions: (1) in West Cumberland on badly contaminated marshy land in a wet season; under these severe conditions the resistance of all varieties broke down: (2) under less severe conditions in Northumberland, where Bangholm and Wilhelmsburgher proved markedly resistant. These less severe conditions are those generally encountered, and the control measures applicable to the area are as follows.

When the full lime requirement of the soil cannot be met, liming is done just before the root crop to improve the tilth and surface drainage of the heavy clay soils, thereby reducing incidence and severity of disease, the use of basic slag for phosphates, and the growing of a resistant variety.

Dry rot (Phoma Lingam) occurs widely and severely; on some farms with short rotations the growing of swedes has been abandoned on account of this disease. On the farm the trouble arises from a combination of affected seed and contaminated dung. Preliminary observations only have been made as yet, in preparation for attempting control on a farm next year by seed treatment and the growing of roots without dung for several years, this necessitating a radical change in general farm practice. One observation showing the possibility of loss from infected seed may be quoted. Seed from two stocks of one variety from the same merchant were grown alongside each other on a field which was not contaminated in any way; one stock of seed gave 60% of diseased roots, the other stock 1%.

Brown heart of swedes. This trouble was first observed in Cumberland in 1931, and was associated with heavy liming of fields near to a free supply of waste lime. Our first field experiments with dung and lime were failures, the reasons not being known until

after the Canadian discovery in 1934 that brown heart was a result of boron deficiency in the soil. Thereafter other trials were made in this area, culminating in 13 field plots on various types of soil in districts from North Cumberland to South Westmorland. An account of this disease and the trials was published in the *Journal of the Ministry of Agriculture* (1939), **45**, 1232. So far as concerns this Northern Province the principal points emerging were as follows. Boron deficiency is not known in Northumberland and Durham; it occurs in the lighter soils of the west. Boron deficient soils do not occur uniformly throughout any district or over any farm, so that routine treatment cannot be recommended. The trouble is eliminated by use of borax or boronated basic slag, at a cost of from 2*s.* 6*d.* to 3*s.* 6*d.* per acre, and by boronite (a dressing ready for application from a manure distributor) at 11*s.* per acre. Part of this problem still remains, namely, the observations of other crops on the treated areas and of the need for treatment in the following swede crop.

Cereal crop problems

At least three-quarters of the cereals in this Province is oats, and since the dressing of seed with mercurial dusts is now widely practised the usual diseases of oats do not cause serious loss. A problem which does arise frequently is a form of grey leaf on land suffering from colliery damage; trials of manganese sulphate dressings often fail to cure the trouble, and it is assumed that other conditions in these defective soils induce this physiological defect in oats.

Barley and wheat are grown to considerable extent in the most suitable districts of Northumberland and Durham, but the fall of arable area from 80 to 30% has correspondingly reduced cereal husbandry. The usual diseases are always with us, but yellow rust and *Fusarium* disease call for special mention. Yellow rust is here favoured by the high condition of land after the long days, and by the use of susceptible varieties such as Standard Red, Squarehead's Master, Wilhelmina, etc. Little Joss is unsuitable for the district, and is grown in one small area only. *Fusarium* disease is not markedly dependent on previous cropping, and is favoured by the sour soils, cold and wet spring weather, and by frequent wet harvests. It attracts most attention in spring when crops fail to respond to favourable growing conditions. The investigations have been concerned hitherto with the species of *Fusarium* and their pathogenicity toward cereals; the outstanding discoveries were the prevalence of two pathogens, *F. culmorum* and *F. avenaceum*, and the occurrence in this country of *Gibberella Saubinetii* and *F. nivale*. The present and future investigations concern the disease under natural conditions in field plots, but of these nothing can yet be said with certainty. *Ophiobolus graminis*, the other foot rot organism, occurs regularly, but to small extent even where two or three cereal crops are taken in succession after long leys. Garrett's recent researches indicate that green manuring well in advance of corn sowing is one of the best means of controlling *Ophiobolus*, and we can now see a reasonable explanation of the relative freedom from this disease in the north, namely a combination of more or less acid soil, successively lower susceptibility of the kinds of cereals grown in succession, and the green manuring by long leys.

Potato crop problems. The chief potato-growing districts lie just around the industrial districts of the Tyne, Tees, and West Cumberland. New seed gives satisfactory results for 3, 4 or 5 years, consequently growers are content to renew stocks from Scotland as required. Spraying against blight (*Phytophthora infestans*) is practised

to a small extent only, the growers contending that it is more economical to risk an occasional bad season than to spray every year.

The problem we undertook in relation to potato husbandry in the north was the production of seed potatoes in the Eden valley where soil and climate are specially suitable. A growers' association now functions automatically, and seed potatoes produced here compare most favourably every year with the best of seed from elsewhere. A development of this work is the improvement of the existing stocks of the "crisp making" variety Bintje; after several years' selection two fixed types have been segregated and are now being grown on a large scale for factory trials. The improvement in these nucleus stocks maintained in Cumberland becomes an advantage also to those who multiply them and grow them on a large scale elsewhere.

Market-garden crops

For reasons previously mentioned market gardening is confined to the districts surrounding industrial centres, and comparatively few types of crops are grown. To avoid misconception it should be mentioned that during the past year advice was given concerning more than fifty different kinds of horticultural plants. Outstanding crops are brassicas, leeks and tomatoes, and these provide some special problems of particular importance in this area. First came the insistent demand for information about leek blight. The leek growers are mainly small gardeners who specialize in exhibition leeks, some of whom have improved and maintained their own stocks for 50 years. Just prior to publication of the investigation of the disease and control measures, Foister, working independently, also ascertained the causal organism and named it *Phytophthora Porri*. Perfect control of the disease is obtained by spraying the plants with Bordeaux mixture, in spite of the fact that the organism persists in the soil and a soil treatment might appear more appropriate. The reasons are given in publications on this subject.

Amongst the brassicas a problem under present investigation is a chlorotic condition resembling a mosaic, but not, according to Dr Kenneth Smith, of virus nature. It occurs throughout a district, causing the dying off of winter and spring greens under frost, and stunted useless heads in cauliflower and broccoli. The investigation is based on soil dressings and exchanged transplants. Awaiting attention is a problem concerning cauliflowers and broccoli of pink colour and bitter taste.

Amongst tomatoes all the usual troubles are encountered, but the outstanding one is root rot associated with *Colletotrichum*. Its prevalence and severity may be linked with the fact that soil temperatures remain low until comparatively late in spring, and growers mistakenly follow the practices of warmer districts. Advice given from Cheshunt is followed, but not always with success, so it is hoped to investigate this problem under local conditions at the Cumberland Horticultural Station in the near future.

The list of problems in the Northern Province would not be complete without mention of the diseases of fine turf as cultivated for golf course and bowling greens. The best of such greens are laid with sea-marsh turf, and the first and best of such turf came from Cumberland. It is appropriate, therefore, that the diseases should be investigated here. The problem in the first place was to ascertain the reasons for the failure of fine turf greens, and this led to the recognition of diseases. Of these there are three common ones, viz. *Fusarium* patch, *Corticum* rust, and dollarspot. For the first

two diseases control measures have been devised and are practised successfully; for the last named satisfactory control has not been obtained—the British organism does not respond to the treatment utilized in the U.S.A. There are instances also of *Rhizoctonia* disease, the American "brown-patch" in Britain, and isolation and comparison of strains are nearing completion. The problem in hand in connexion with these diseases is to devise one treatment for all to obviate need of diagnosis and differential treatments. To this end some 20 chemicals and proprietary articles have been examined as to their toxicity toward, or capacity for, inhibiting the growth of the various fungi and their different strains. This laboratory work is nearly completed and will later be carried into practical trials.

It may be gathered from the foregoing outline that, although many agricultural and horticultural crop plants are scarcely or not at all grown in the north, there is ample scope for investigation of local problems and for bringing mycological science into practical application.

REVIEWS

Diseases of Fruits and Hops. By H. Wormald. Pp. 290. London: Crosby Lockwood and Son, Ltd. 1939. 17*s.* 6*d.*

For almost the whole period of his scientific life Dr Wormald has been engaged in research on diseases of fruit trees and hops, and, since 1923, he has been in charge of pathological work at the East Malling Research Station. His experience of these problems must be almost if not quite unique and there can be but few growers' troubles of which he does not possess first-hand knowledge. His own numerous publications have contributed notably to our understanding of these problems and he has now increased our debt by the production of this valuable work. This book, together with Massee's *The Pests of Fruits and Hops* (see *Ann. appl. Biol.* 1937, **24**, 667) forms a splendid survey of the fruit-growers' troubles. The book is written essentially for the grower and aims at describing symptoms rather than pathogenic organisms. Its arrangement facilitates identification of the diseases which are grouped according to the host plants, and then under the various organs on which the symptoms are to be seen.

Ch. I is introductory dealing with factors conducive to health or disease in plants, and Ch. II is a brief but clear and practical account of fungicides and their application. Some diseases such as apple scab or apple canker are restricted to particular hosts, but others such as crown gall or grey mould affect a number of different plants. These more general diseases are dealt with in Ch. III although they are mentioned again under hosts, with special reference to particular symptoms or the effect on those plants. The following ten chapters are then devoted to the more special diseases of the large and small fruits, two chapters being given to the apple, one to pear, quince and medlar, two to the stone fruits, three to bush fruits, one to the strawberry, and one to the grape vine, fig, mulberry, walnut and cob nut. Hops are not in the same category as the fruits mentioned above, but Dr Wormald was wise in his inclusion of Ch. XIV which is an excellent account of diseases of this plant. A final and valuable chapter is devoted to fruit tree diseases such as fire blight, internal cork, etc., which are important in other countries but not yet recorded here.

Appended to the discussions of many diseases are selected references, and the book concludes with indexes to popular names and control measures, to scientific names, and to authors. In addition to twenty-four text-figures, one or two of which are not quite up to standard and in a few of which no magnification is stated, the work is illustrated by forty plates each containing from four to six photographs. The latter, taken by Dr Wormald himself, or by Miss Cornford under his supervision, are one of the finest series of illustrations yet published in any volume on plant diseases. The work is written in a simple, clear style and there can be no excuse for any grower failing to recognize particular diseases described; control measures advised are practical and feasible. In less skilful hands a book planned as is this would inevitably have contained much repetition but Dr Wormald has largely been successful in avoiding this.

It is difficult to find points of serious criticism in the work. A tabular key based on symptoms might have been a useful addition, but the author's arrangement and descriptions are so clear that it is really unnecessary. Other points noted may be mentioned. The author is a little inconsistent in giving spore measurements sometimes as averages, sometimes as maximum and minimum. In his citation of references he does not adopt the standard abbreviations as given in the *World List of Scientific Periodicals* and his own abbreviations are not consistent. Although the book is not over-burdened with references some of those cited seem unnecessary, whereas others that one might expect are omitted. On many of the plates there is a considerable amount of wasted space.

The book will be useful not only to growers, but also to students as a complementary volume to standard textbooks of plant diseases. It contains a foreword by Dr Pethybridge and, very appropriately, is dedicated to Prof. E. S. Salmon.

WILLIAM B. BRIERLEY.

Nytteplanter. By K. Gram, H. J. Jensen and A. Mentz. Pp. v+503. Copenhagen: Messrs Gyldendal, Ltd. 1938. 9.75 kr.

During the last two decades valuable works dealing generally with the field of economic plants have been written by American, British, French, and German authors but this is the first Danish treatise to be published.

The contents of the book are arranged in five main divisions: I, Food plants (pp. 233; starch; oil-nuts; sugar; roots, vegetables and salads; fruits; forage): II, Aromatic plants and stimulants (pp. 72; including beverage plants): III, Medicinal, drug and poisonous plants (pp. 50): IV, Technical plants and products (pp. 118; fibre; cork and timber; charcoal; rubber; oil; wax; saponin, soda, iodine, etc.; gum; scent; tannin; dye): V, Plants used in conserving or improving soil (pp. 12).

The plants, their culture and economic uses, receive brief but adequate description, and the text is illustrated by 300 excellent photographs and line-drawings. There is a bibliography of 72 citations and an index.

WILLIAM B. BRIERLEY.

Poisonous Plants of the United States. By W. C. Muenscher. Pp. xvii +266. New York: The Macmillan Co. 1939. 15*s.* 0*d.*

Part I of this book deals with the nature and classification of poisonous plants and occupies only 18 pages. It is merely a very brief summary of the generalities of the subject—the chemical nature, properties, and physiological action of the toxic principle, and the conditions under which poisoning is produced, and then brief accounts with lists of plants causing dermatitis, or photosensitization, cyanogenetic plants, introduced poisonous ornamental plants, plants producing poisonous seeds, seleniferous plants, plants producing undesirable flavours in milk and milk products, and plants causing mechanical injury. The author must have gone to considerable trouble to review and summarize the data of this portion of the subject, and it is a great pity that he did not amplify his consideration of the problems, since an up-to-date and comprehensive survey of these more fundamental issues would have been extremely valuable.

Part II (pp. 19–239) is an accurate and concise account of poisonous plants arranged by family according to Engler's system, about 400 species representing 68 families being included. Fungi are omitted but all the vascular plants of the U.S.A. that are known to cause poisoning when eaten, by contact, or by mechanical injury, to man or animals, are included. In general each plant is treated as to names, description, distribution and habitat, poisonous principle, conditions of poisoning, symptoms; and in a few cases simple remedies are indicated or references to treatment are cited. The consideration of each species is brief and to the point and, as in the author's previous book *Weeds* (see *Ann. appl. Biol.* 1936, **23**, 662), the text is illustrated by numerous original and beautiful full-page line drawings by Mrs Helen Hill Craig. There is a bibliography of 231 references and an index.

The book is naturally in very large part based upon the literature of the subject, but it is an extremely good compendium, and it will serve as a useful companion volume to the recent second edition of Long's *Poisonous Plants on the Farm.*

WILLIAM B. BRIERLEY.

Introduction to the Botany of Field Crops. By Prof. J. M. Hector. (South African Agricultural Series, Vol. 16.) Vol. I: Cereals. Pp. xii + 478 + xiii–xxxiv. Vol. II: Non-Cereals. Pp. viii + 479–1128 + ix–xxxiii. Johannesburg: Central News Agency, Ltd.; London Agents: Messrs Gordon and Gotch, Ltd. £3. 10*s.* 0*d.* net per set.

In these two volumes Prof. Hector deals with those families that contain plants cultivated as field crops in temperate, tropical, and sub-tropical countries.

Vol. I is devoted to the cereals and includes chapters on oats, wheat, rye, barley, rice, millets, sugar-cane, sorghum, and maize, prefaced by a chapter describing the general characters of the Gramineae. In each chapter the author describes the growth and development of the cereal under consideration, its detailed anatomy, and its genetics, and discusses the classification of the species and cultivated forms belonging to the genus, and also the origin of the cultivated kinds. At the end of each chapter there is an extensive bibliography including work published up to the end of 1935 and some of the 1936 publications, but, as the author remarks in his preface, "no attempt has been made to deal adequately with the literature of 1936". Considering the difficulty of obtaining certain literature in the Union of South Africa, these bibliographies are excellent, e.g. there are over 400 references listed at the end of the chapter on wheat and nearly 300 at the end of that on maize.

Vol. II includes the families containing the principal root, forage, fibre, and vegetable crops of the world. The longest chapters are those on the Malvaceae, Solanaceae, Leguminosae, and Cruciferae families, the other chapters dealing with the families Liliaceae, Moraceae, Polygonaceae, Chenopodiaceae, Linaceae, Umbelliferae, Convolvulaceae, Cucurbitaceae, and Compositeae. In his treatment of each family, the author describes the general characters very briefly, and follows this description by a classification of the genera and species of economic importance. He then describes the development, anatomy and genetics of the more important members of each genus and gives a brief account of those of lesser importance. The origin of the cultivated forms and their economic uses are also discussed. As in the volume on cereals each chapter is followed by an extensive bibliography. At the end of each volume there are very useful plant and author indexes and a very good general index. The books are well printed and profusely illustrated, most of the illustrations being copied from those of the original publications.

Agricultural botanists all over the world will be extremely grateful to Prof. Hector for these volumes, in which he has given a co-ordinated and masterly account of the immense amount of work which has been done since 1900 on the botany of cultivated plants. They will also be thankful for the references to the widely scattered literature on the subject. This work, also, has a much wider appeal than to agricultural botanists for the chapters on the Solanaceae, Cruciferae, and Chenopodiaceae families include not only plants that are usually grown on farms, but garden and market garden plants such as the tomato, egg plant, cabbages of all kinds, radish, sea kale, chard and spinach. In other chapters there are descriptions of onions, asparagus, melons, cucumbers, lettuce, artichokes, and other garden crops. The inclusion of these plants is of great value to the horticultural botanist for the literature on them is widely scattered and difficult to obtain. "Pure botanists" will also find these volumes very useful and they might with advantage use much of the material for courses in "pure botany" since our knowledge of many of the crop plants is far more detailed and extensive than that of many plants of theoretical interest which figure so largely in these courses. To the agricultural student who hopes to obtain a colonial post, the chapters on tropical and sub-tropical plants are most helpful.

The treatment of one or two of the families, particularly the Chenopodiaceae, is somewhat disappointing, the references to sugar-beet being distinctly out of date. Also, the author might with advantage have included descriptions of the external morphology and illustrations of many of the plants, instead of assuming the reader to have a knowledge of their appearance. These, however, are minor criticisms of a

work of this size, and Prof. Hector is to be congratulated on the production of a work of such a high standard of quality and usefulness.

ADELA G. ERITH.

Commercial Fruit and Vegetable Products: a Textbook for Student, Investigator and Manager. By W. V. Cruess. 2nd Edition. Pp. x + 798. London: McGraw-Hill Publishing Co., Ltd. 1938. 36*s.* 0*d.*[1]

The first edition of this well-known American work was published in 1924. Since then important advances have been made in the technology and underlying sciences of the industries, the freezing storage of vegetables and fruits has attained industrial importance, and the prohibition law of the U.S.A. has been repealed.

Every chapter of the new edition shows emendation, the chapters on vitamins, canned-fruit spoilage, tomato products, and canning, have been completely revised, and there are additional new chapters dealing with plant pigments, enzymes of fruits and vegetables, freezing storage of fruits and vegetables, and the making of wines. The chapter bibliographies have in most cases been brought up-to-date. Ch. 1 dealing with microorganisms in relation to fruit and vegetable storage still needs some expert mycological attention.

The book is a comprehensive survey of an important and very wide field in which knowledge and application are advancing rapidly. The production of especially suitable varieties of fruits and vegetables for canning, drying, etc., is essentially a problem for the geneticist; pests and diseases in the growing plants and harvested crops demand the entomologist and plant pathologist; the processes and conditions of preservation are problems for the plant physiologist; and problems of deterioration and spoilage are work for the mycologist and bacteriologist. The whole series of industries is based essentially on applied biology, and applied biologists should play a much larger part in these developments than they do at present, and they should receive from the industries a very much larger measure of support.

WILLIAM B. BRIERLEY.

The Rape of the Earth: A World Survey of Soil Erosion. By G. V. Jacks and R. O. Whyte. Pp. 313 and 47 Plates. London: Faber and Faber, Ltd. 1939. 21*s.* 0*d.*

A few months ago the authors of this book published a short factual survey of soil erosion in various countries (see *Ann. appl. Biol.* 1938, **25**, 874) and they have now produced, under a strikingly apt title, a larger and more comprehensive work of quite outstanding importance.

Much of the world is, to a large extent, governed by or from Western Europe, and since in this region soil erosion is practically negligible, the subject has received little recognition. Only a few people have heard of it, and fewer still have any clear idea of its nature and importance; yet, as the authors state, "Soil erosion is altering the course of world history more radically than any war or revolution. Erosion is humbling mighty nations, re-shaping their domestic and external policies and once and for all it has barred the way to the El Dorado that a few years ago seemed almost within reach." Life on this earth depends upon a tenuous skin of soil, and over vast areas this skin is being attacked by "a contagious disease spreading destruction far and wide irrespective of private, county, state or national boundaries". "The only sure foundation upon which a superstructure of civilization can be built is a stable soil" and the very foundation of civilization and even of life itself is being blown into the air or washed into the sea. Of South Africa, General Smuts has said "Erosion is the biggest problem confronting the country, bigger than any politics" and this is equally true of many other vast regions.

In its catastrophic menace soil erosion is a thing of the last few decades and it is increasing at terrifying speed, but it is not an Act of God like an earthquake; it is

[1] As from 2 October, 1939, the McGraw-Hill Publishing Co., Ltd. have raised the net prices of their books by 10 % throughout the whole of their catalogue.

man made and it can be stopped. "It is not so much the damage already done that matters as the final and inevitable disaster to civilization that will occur if the contagion is allowed to spread until it is uncontrollable. A war-scarred country can be restored to prosperity in a few years; a field stripped of its soil is finished, at least so far as providing for the living generation is concerned, but can continue to spread destruction over other land." "We now know fairly precisely what agricultural, pastoral, forest and engineering principles must be adopted to stop the earth from rotting away beneath our feet", but because of established conditions and practices "we cannot, or dare not, apply them forthwith on a scale commensurate with the gravity of the situation" save at the risk of "a social and political revolution". "Great changes will have to be made in the kinds and quantities of crops produced, exported and imported by different countries, internal and external trade relations and policies will be affected, and perhaps most significant of all, the conditions of land tenure and occupancy upon which the social structure of a civilized community is founded, will have to be re-defined. Erosion control, acknowledged in many rapidly developing countries as the most vital problem confronting them, means going back to the beginning and re-building human society in a frame whose shape is determined by the intrinsic nature of the soil and is independent of immediate economic or political considerations."

To many, perhaps most people, especially if they have not travelled in eroded lands, all this may seem wild exaggeration, but the authors support their statements with a wealth of data which, cumulatively, is almost overwhelming. Also, it may be objected, that large scale industrial processes of synthesizing food stuffs, or a huge development of water culture methods (Hydroponics) may, in the future, provide all the food that man requires and render soil farming unnecessary. But to many of us it would seem fairly clear that industrialism itself suffers increasingly from much the same kind of disease as does the soil, and it is very clear indeed that water culture methods have a long, long way to go before they can supply even a tithe of our food stuffs. In any case it would, from every point of view, be infinitely better to preserve our mother earth by controlling soil erosion and to use synthetic and hydroponically produced food stuffs as accessory. What *must* be recognized is that the soil is the world's most valuable natural resource, and that in formulating agricultural and social policies the rights of the land must be placed first.

The authors' approach is based on two simple theses which in essence are, first, that Western European civilization is based on an agriculture which has slowly evolved on the conquest of the forest in humid temperate regions and, secondly, that this agriculture, with all its concomitants, has been transferred to prairie, semi-arid and tropical regions to which it is proving quite unsuitable; erosion is one result of this unsuitability. "The agriculturalist's main object is to live *off* rather than *on* the land, to bleed his soil for the sake of distant towns, and himself to be maintained and enriched by their produce. Farming has become an industry and irreplaceable soil fertility an industrial commodity to be bought and sold and transferred to the ends of the earth as easily as any other commodity." "Cultivation of the soil became a means of wealth instead of a mode of life. The more that could be got out of the soil and the less that had to be put back, the better for the cultivator."

The book is a composite production, the more factual chapters being written by Dr Whyte, and the more speculative, i.e. economic, and social chapters by Dr Jacks, but the work coheres as a unity. Whilst one is not compelled to accept the detailed social and economic conclusions at which the authors arrive, probably every reader of this book will feel that they have pictured a condition of things the full and immediate recognition of which is vital to human life and welfare over vast regions of the globe, and that they have discussed the social and economic implications of this condition in an interesting and enlightening manner. All thinking men, not only in eroding countries but in Western Europe, whatever their political and economic views, must face up to this situation. The problem for which the last and present generation is largely responsible cannot be left for future generations to solve; we know what has to be done and it is imperative that we do it whilst it still remains possible.

WILLIAM B. BRIERLEY.

Science in Africa: A Review of Scientific Research relating to Tropical and Southern Africa. By E. B. WORTHINGTON. (Issued by the Committee of the African Research Survey under the auspices of the Royal Institute of International Affairs.) Pp. xiv+746. London, New York and Toronto: Oxford University Press. 1938. 10*s.* 6*d.*

This volume forms part of the African Research Survey conducted under the general direction of Lord Hailey. Dr Worthington, who had contributed valuably to knowledge of the biology and fisheries of East African lakes, was asked to prepare a report on scientific research and technical services bearing on African conditions and development, and to this end visited Nigeria, Dahomey, Togoland, the Gold Coast, Sierra Leone, the Gambia, Senegal, and the French Sudan. He writes, therefore, with unusual first-hand knowledge of African conditions.

The book covers an enormously wide field, the several chapters dealing with general problems of research, surveys and maps, geology, meteorology, soil science, botany, forestry, zoology, fisheries, entomology, general agriculture, crop-plants, plant industry, animal industry, general health and medicine, human diseases, health and population, and anthropology. The author does not pretend to knowledge or opinion outside his own study or experience, but simply states the views of experts as objectively as possible without attempting to assess the value of rival theories. The material is arranged more or less uniformly in each chapter under the headings of introduction, organization, and results, and territorial arrangement is followed when subjects lend themselves to such treatment. In consequence, although the book contains an enormous mass of data, it is extremely easy to use. The more important research up to 1936 is included, with the exception of medical research which is more or less complete only to the end of 1934. The various chapters of the volume in draft were submitted for criticism and suggestion to numerous specialists listed on pp. 615–25, but the volume is essentially the personal achievement of Dr Worthington. The sources of information are given in a classified and extremely useful bibliography occupying pp. 627–91, and there is an excellent index.

It is impossible in a brief notice of a volume so wide in its range to enter into any detail, or in any way to do justice to the quality of the work. Browsing through the volume and reading critically chapters and sections dealing with subjects in which one is particularly interested, one is impressed not only by the wide scope and comprehensiveness of the work, but by the balance and fairness of the author's treatment, and most of all, perhaps, by the insight he shows into problems and research outside his own special knowledge and experience. The book gives one a remarkably clear picture of the state of scientific research in Africa, of its organization, of the problems under investigation and of those partially or wholly solved, of the infinite number of problems to be faced, of the way in which the problems integrate with each other, and of the difficulties created by their interdependence. At the same time the book is no dry-as-dust catalogue; Dr Worthington writes well and his book is extremely interesting and readable, being at once both informative and suggestive.

As the author states, "A development based on a real understanding of Africa's potentialities has hardly yet begun, and will be impossible until the necessity of scientific knowledge is recognised." Few books could do as much to bring about this recognition as *Science in Africa*, and one can only be grateful for Dr Worthington's achievement, for there are very few scientific workers who would have had the courage to assay his task or to complete it so successfully.

Finally, one may perhaps draw attention to the fact that through the generosity of the Carnegie Corporation of New York, this beautifully produced volume of 746 densely packed pages, illustrated by eight plates, four text-figure maps, and a large coloured folding map with insets, is obtainable at the remarkably low price of 10*s.* 6*d.*

WILLIAM B. BRIERLEY.

Health and Nutrition in India. By N. GANGULEE. Pp. vii + 337. London: Faber and Faber, Ltd. 1939. 15*s.* 0*d.*

Dr Gangulee was formerly Professor of Agriculture and Rural Economy in the University of Calcutta, and served as a Member of the Royal Commission on Indian Agriculture. The thesis of his book is that India's main problem is one of health and nutrition in relation to socio-economic conditions, and that the limiting factors are poverty and shortage of food. The problem is first stated in general terms, and then the necessary basis for its consideration is given in an account of modern nutritional science and the consequences of dietary deficiencies. The conditions of public health in India are then described with special reference to the main theme, and a survey is made of Indian foodstuffs, and of the diets of various Indian communities. These six chapters thus describe the general facts and principles and their exemplification in India. The author then discusses what is being done in certain other countries with regard to analogous situations and, finally, considers the problem in India and makes concrete suggestions for action.

In the factual chapters the author is admirably clear and concise—chapters II and III, for example, contain one of the best simple accounts I know of the principles of modern nutritional science. But Prof. Gangulee's volume is not a textbook of dietetics; it is a thoughtful and constructive essay on a major human problem, the mental and physical health of nearly 400 million people. And in writing of this tragedy Prof. Gangulee is not merely a scientist writing of matters of fact but a man writing of his own country and of his own people. His pen distils a rich humanity, a sustained enthusiasm, and a flame of idealism tempered only by clarity of vision, unusually wide knowledge, and a wealth of sobering experience. It is not possible here to give any idea of the author's treatment of his subject, or of the richness of his book in scientific data, constructive thought, and practical suggestion. The book is an essay in applied biology, applied biology on an epic scale, and the author shows clearly how the application of biological knowledge we already possess could ameliorate the lives of one-fifth of the world's population. No-one who cares for India dare neglect this courageous book.

WILLIAM B. BRIERLEY.

An Introduction to Modern Genetics. By C. H. WADDINGTON. Pp. 441. London: George Allen and Unwin, Ltd. 1939. 18*s.* 0*d.*

Dr Waddington's own researches have lain in the field of experimental embryology rather than genetics but, in many ways, this adds to the value and interest of his work since he is able to view data and relations from a wider angle than seems to be possible by most professional geneticists. His aim is not so much merely to add another formal textbook of genetics to the library already in existence as to integrate genetics with organic development and evolution, a more useful and difficult task. As he says in his Preface: "I want to urge that the connection between genetics and the other branches of biology, such as cytology, embryology, the study of evolution and of the biochemical nature of cell constituents, is much closer than is often admitted, and that the boundaries between these subjects deserve less attention than is usually paid to them."

The subject-matter of the book is divided into five parts. Part I deals with formal genetics—the fundamentals of Mendelism, modifications of the chromosome cycle, the behaviour of individual chromosomes, their linear differentiation, and their mechanics. All this forms the modern groundwork of the subject and occupies about one-third of the book. As a brief presentation of the general situation to-day these chapters seem to me quite excellent, but since schools of thought are now growing up in genetics there will almost certainly be geneticists who will find grounds for cavil with both the detail and the balance and perspective of the author's treatment. Incidentally, the brief summary on p. 27 does not correspond with the actual chapters.

Part II deals with genetics and development and contains chapters on genes and development, the interaction of genes and its effects, gene controlled processes, the genetic control of pattern, and sex determination. It is in this part especially that the author's developmental viewpoint and his wide range of knowledge have full scope, and his consideration of these difficult problems is illuminating.

Part III is devoted to genetics and evolution, contains an introductory chapter on the processes of evolution, and then two stimulating if somewhat controversial chapters on the genetic nature of taxonomic differences and evolutionary mechanisms. The relation of cytogenetics to organic evolution is often either evaded by genetical writers or dealt with quite inadequately, and Dr Waddington's brief but masterly discussion of this problem is, therefore, all the more welcome.

Part IV which deals with genetics and human affairs contains only two chapters. The first is a very summary account of animal and plant breeding and, although one sympathizes with the author's difficulty in spelling "*Phytophthora*", this chapter seems to me far below the general standard of the book. The chapter on human genetics is, however, quite brilliant and one can only wish that some of the more rabid eugenists and racialists could be induced to read it.

To more general readers Parts III and IV will be by far the most interesting in the book. In Part V the author returns again to more abstruse matters and discusses the nature of the gene. Goldschmidt's somewhat pessimistic views are regarded as "extremely premature" and the numerous alternative hypotheses are clearly and fairly stated.

A useful Appendix describes laboratory methods for class work on *Drosophila*. The book opens with a synoptic contents and concludes with an excellent combined bibliography and author index (which contains a number of misprints) and an inadequate subject index. It is illustrated by 5 plates and 160 text-figures, many of the latter being over reduced, and it is bound in a very startling cover.

The author writes in a condensed but clear and interesting style and, although for general scientific readers the book will not always be easy going, the effort will be found well worth while. Not only in all fields of biology but in the more general work-a-day world the data and implications of modern genetics are so vitally important that it is to be hoped that this book will reach a very wide public. Not only is it remarkably up-to-date but it seems to me the best introduction to genetics in its wider biological relations that has yet been published.

WILLIAM B. BRIERLEY.

Earth's Green Mantle. By S. MANGHAM. Pp. 332. London: The English Universities Press, Ltd. 1939. 10*s.* 6*d.*

This is an excellent and unusually comprehensive survey of modern plant science written for the general reader. Although the author's choice of material and points of view are essentially those of a teacher of botany in an academic institution he shows considerable sympathy with the more applied aspects and it is interesting that in his final chapter, headed "Progress and Prospects", his viewpoints and material are entirely applied. The book opens with an effusive foreword by Sir Arthur W. Hill, and closes with a somewhat unsatisfactory list of books suggested for further reading, and an Index. It is illustrated by forty-two text-figures and forty plates, each of the latter containing from one to eight excellent photographs which, in many cases, suffer from over-reduction.

The book is accurate, up-to-date, and interesting, for the author has performed his task well. It is written in a non-technical but rather pedantic style with a tendency to the use of such language as "excavatory operations", "the revelations of this optical servant of the scientist", "the resources available for probing life's mysteries", and even "How prodigal Nature is!"

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